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(54) Title: CELLS EXPRESSING ANTI-Fc RECEPTOR BINDING COMPONENTS		
(57) Abstract		
<p>Cells transformed to express on their surface a component which binds to an Fc receptor of an effector cell are disclosed. Also disclosed are expression vectors used to transform the cells. Once transformed, the cells bind to effector cells via the Fc receptor of the effector cell to stimulate an effector cell mediated immune response.</p>		

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CELLS EXPRESSING ANTI-Fc RECEPTOR BINDING COMPONENTS

Background of the Invention

Antigen-specific antibodies increase the uptake of antigen, resulting in enhanced T cell activation (Chang, T.W., *Immunol. Today*, 6:245, 1985). Antibody:antigen immune complexes bind to Fc receptors present on the surface of antigen presenting cells (APC), such as macrophages. These complexes are then internalized and the antigen is processed and presented in the context of MHC-encoded molecules to antigen-specific T cells. Thus, the presence of antigen-specific antibodies can reduce the level of antigen required to activate T cells (Gosselin, E.J., *et al.*, *J. Immunol.*, 149:3477, 1992).

Receptors for the Fc portion of IgG (FcγR) have been characterized at the molecular level. Human monocytes and macrophages express three major classes of FcγR, identified as FcγRI, FcγRII, and FcγRIII. Targeting tetanus toxoid to either FcγRI or FcγRII on monocytes reduces the concentration of antigen required to stimulate T cell proliferation *in vitro* by a factor of 100-1000 (Kovacsovics-Bakowski, M., *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:4942, 1993). Therefore, targeting antigen to specific FcγR on APC can dramatically decrease the amount of antigen required to stimulate a specific T cell response.

Following phagocytosis by a monocyte or a macrophage, antigens can be processed and presented to cytolytic T lymphocytes (CTL; Falo, L.D., *et al.*, *Nat. Med.* 1:649, 1995). It has been shown that mice immunized with an iron particle coupled to a tumor antigen are protected from tumors expressing that tumor antigen (Kovacsovics-Bankowski, M., *et al.*, *ibid*). Therefore, by inducing monocytes or macrophages to phagocytose tumor antigens, protective immunity to tumors can be induced.

Summary of the Invention

The present invention provides a cell which is transformed to express on its surface a component which binds to an Fc receptor of an effector cell. In particular embodiments, the Fc receptor binding component on the cell is an antibody or portion thereof, such as a single chain Fv fragment, which binds to the Fcα receptor or the Fcγ receptor present on effector cells. The transformed cell is thus targeted to the effector cell via the Fc binding component, and can be used as a vehicle to increase an effector cell-mediated immune response, such as cell lysis and phagocytosis, against an antigen associated with the cell. Examples of target antigens include, but are not limited to tumor antigens, such as HER-2 *neu*, TAG 72, carcinoembryonic antigen and gastrin releasing peptide receptor, and components from pathogens, such as a virus, fungus, protozoan, or bacterium.

The anti-Fc receptor binding component is produced recombinantly within the target cell in a manner which causes it to be expressed on the surface of the cell. In a preferred embodiment, the anti-Fc receptor binding component is able to bind an Fc receptor of an effector cell without being blocked by endogenous antibody, e.g., IgG or IgA. In another preferred embodiment, the anti-Fc receptor binding component is an antibody or antibody fragment, such as an IgA, IgG, IgM, IgE, or fragment thereof (e.g., Fab, Fab', F(ab')₂, Fv, or single chain Fv fragment).

In a particular embodiment of the invention, the anti-Fc receptor binding component is expressed recombinantly as a fusion protein associated with the membrane of the target (transformed) cell. For example, the anti-Fc receptor binding component can be expressed in the cell as a fusion protein together with a transmembrane protein or portion thereof (e.g., transmembrane domain of the protein). In such embodiments, a preferred anti-Fc receptor binding component comprises an anti-Fc receptor antibody or antibody fragment, such as humanized anti-FcγR antibody 22 (H22) having the ATCC deposit number CRL 11177, anti-FcαR monoclonal antibody A77 (Monteiro et al. (1992) *J. Immunol.* 148:1764). or a single chain Fv fragment of H22 or A77. In a particularly preferred embodiment, the anti-Fc receptor binding component is expressed as a fusion protein made up of a single chain Fv fragment of H22 or A77 and a transmembrane protein (e.g., the transmembrane domain of the platelet derived growth factor receptor).

Accordingly, in addition to cells that express Fc receptor binding components, the present invention also provides vectors and expression plasmids, such as anti-FcγR pJG717 (SEQ ID NO:1) and anti-FcαR pJG718 (SEQ ID NO:2), which can be used to transform cells so that they express components which bind Fc receptors.

The present invention further provides a method of increasing an immune response in a subject using transformed cells of the invention. The method involves contacting the transformed cell with an effector cell in the presence of a lymphocyte, (e.g., a T cell or B cell). In one embodiment, the effector cell is treated with an agent that increases expression of Fc receptors on the surface of the effector cell. Suitable agents include cytokines, such as granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), γ-interferon (IFN-γ), tissue necrosis factor (TNF), and combinations thereof.

Cells of the present invention can be transformed to express Fc receptor binding components either *in vivo* or *ex vivo*. In a preferred embodiment, the cell is transformed *ex vivo* and is then administered to a subject *in vivo*. Following administration, the cell binds to an effector cell via an Fc receptor of the effector cell (e.g., Fcγ receptor, an Fcα receptor, an Fcμ receptor, or Fcε receptor). In particular embodiments, the Fc receptor is

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an Fc γ receptor selected from Fc γ RI, Fc γ RII, and Fc γ RIII. Alternatively, effector cells can be taken from the subject by, for example, apheresis, modified with cytokines to optimize antigen presentation and Fc receptor functions *ex vivo*, combined with cells of the present invention transformed to express Fc receptor binding components, and then
5 returned to the subject.

Among various other uses, transformed cells of the present invention can be used to induce a specific immune response against an antigen associated with the transformed cell, such as a T lymphocyte mediated immune response. Accordingly, transformed cells of the invention can be used to achieve protective immunity against selected antigens,
10 e.g., as vaccines. When targeting tumor cells, for example, the present invention provides the distinct advantage in that no particular tumor antigen need be known or selected for targeting. This is because the entire tumor cell itself is transformed to express a component which binds to an effector cell via an Fc receptor to cause killing of the tumor cell. This is of great benefit since many tumors do not have defined antigens
15 for targeting.

Brief Description of the Drawings

Figure 1 is a map of the expression vector pJG717 encoding a fusion protein made up of the platelet derived growth factor receptor transmembrane domain (TM) and
20 a single chain Fv fragment of anti-Fc γ RI antibody H22. This fusion protein is referred to as H22-TM. As shown on the map, CMV Pr is the CMV promoter/enhancer; L is the Murine Ig kappa-chain V-J2-C leader sequence; HA is the hemagglutinin A epitope; myc is the *myc* epitope; TM is the platelet derived growth factor receptor transmembrane domain; BGH pA is the bovine growth hormone polyadenylation signal; ColE1 ori is the
25 ColE1 origin of replication; Tk pA is the thymidine kinase polyadenylation site; Neo is the neomycin/kanamycin resistance gene; SV40 ori/Pr is the SV40 origin and promoter; amp is the ampicillin resistance gene; and fl ori is the fl origin.

Figure 2 is a FACS scan showing expression of H22-TM on the surface of transformed cells of mouse tumor cell lines. The top panel shows a FACS scan of NSO
30 (mouse myeloma) cells carrying pJG717, and the bottom panel shows MTC (mouse thyroid medullary carcinoma) cells carrying pJG717.

Figure 3 is a graph showing H22-TM directed cytotoxicity as percent lysis of chromium-labeled target NSO cells transformed with pJG717, granulocyte-colony stimulating factor (G-CSF)-treated granulocytes as the effector cells, at varying
35 effector:target ratios.

Figure 4 shows the nucleotide sequence of pJG717 and the corresponding amino acid sequence of the H22-TM coding region within the plasmid. Also shown are restriction sites, as indicated on the pJG717 map shown in Figure 1.

Figure 5 is a graph showing ADCC of H22-TM expressing 653 myeloma cells by activated Granulocytes. Granulocytes were purified from normal whole blood and cultured overnight with G-CSF and IFN- γ . The effector cells were combined with ^{51}Cr -labeled untransfected myeloma cells or 653-22TM cells at an effector to target ratio of 50:1. H22 F(ab')₂ or A77F(ab')₂ was added in excess to demonstrate specific blocking of the ADCC. Results are shown as % lysis of H22-TM expressing 653 myeloma cells.

Figure 6 is a graph showing ADCC of H22-TM expressing 653 myeloma cells by human macrophages. Monocytes were purified from normal donors and differentiated into macrophages in the presence of IFN- γ . The effector cells were combined with ^{51}Cr -labelled untransfected myeloma cells or 653-22TM cells at an effector to target ration of 30:1 and incubated for 3.5 hours. H22 F(ab')₂ or A77F(ab')₂ was added in excess to demonstrate specific blocking of the ADCC. Results are shown as % lysis of H22-TM expressing 653 myeloma cells.

Figure 7 is a graph showing phagocytosis of H22-TM expressing murine carcinoma cells (MTC) by human macrophages. Phagocytosis was evaluated by a two-color flow cytometry assay. Macrophages were cultured from monocytes in media containing M-CSF and IFN- γ . MTC cells were labeled with PKH-26 dye (Sigma) and cultured with macrophages in 24 well plated for 18 hours. The macrophages were stained with CD14-FITC before analysis of samples with a FACScan. The % phagocytosis was determined by the formula: (number of dual positive cells) / (total number of red cells) x 100%. The effector to target ratio was 20:1.

Figure 8 is a graph showing induction of cytokines by H22-TM expressing murine myeloma cells (653). Monocytes were purified from normal whole human blood and allowed to adhere to plastic tissue culture plates. The monocytes were co-cultured with 653 cells or H22-Tm expressing 653 cells (653-22TM) at an effector to target ratio of 5:1. Media was removed from the cultures at 0, 4, and 21 hours and measured for the presence of cytokines by commercially available kits.

Figure 9 is a map of the expression vector pJG718 encoding a fusion protein made up of the platelet derived growth factor receptor transmembrane domain (TM) and a single chain Fv fragment of anti-Fc α R antibody A77. This fusion protein is referred to as A77-TM. The genetic regulatory control elements shown on the map correspond to those shown in Figure 1.

Figure 10 shows the nucleotide sequence of pJG718 and the corresponding amino acid sequence of the A77-TM coding region within the plasmid. Also shown are restriction sites, as indicated on the pJG718 map shown in Figure 9.

Figure 11 is a FACS scan showing expression of A77-TM on the surface of transformed myeloma cells. The NSO-A77-TM transformed cell line (77-D6) was incubated with soluble Fc α R (5 μ g/ml) for 90 min. at 4°C. After washing the cells, IgA was added (20 μ g/ml) was added for 90 min. at 4°C. The cells were washed again, and the IgA bound to cells was detected by a goat anti-human IgA-phycoerytherin probe. Panel A shows cells reacting only with probe, Panel B shows cells reacting with soluble Fc α R and probe, Panel C shows cells reacting with IgA and probe, and Panel D shows cells reacting with soluble Fc α R and IgA and probe.

Detailed Description of the Invention

The present invention is described herein using the following terms and phrases which shall be understood to have the meanings provided below.

The term "subject" means any mammal (e.g., human or non-human) possessing leukocytes capable of responding to antigenic stimulation. A "patient" means a human subject.

The term "leukocyte" refers generally to a white blood cell and includes all classes of white blood cells. Leukocytes include cells from three lines of development: myeloid, lymphoid, and monocytic cells. A "lymphocyte" is a white blood cell formed in lymphatic tissue (lymph nodes, spleen, thymus, tonsils, Peyer's patches and sometimes in bone marrow, and includes B lymphocytes and T lymphocytes. A B lymphocyte is responsible for the production of immunoglobulins and expresses immunoglobulins on its surface. A T lymphocyte is responsible for cell-mediated immunity and can be further divided according to function, such as helper, suppressor, and cytotoxic T cell.

The phrase "component which binds to an Fc receptor" includes any agent capable of binding to an Fc receptor on an effector cell, such as a protein or protein fragment that binds specifically to an Fc receptor determinant. The component can be an antibody or antibody fragment as defined herein, and includes an engineered antibody such as a humanized or a chimeric antibody, a Fab fragment consisting of the V_L, V_H, C_L and C_{H1} domains; an Fd fragment consisting of the V_H and C_{H1} domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 *Nature* 341:544-546) consisting of a V_H domain; an isolated complementarity determining region (CDR); and an F(ab')₂ fragment, a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region.

The component can also be a peptide mimetic which mimics the binding of an antibody or antibody fragment as defined herein. The component can also be a chemical compound, such as a cyanidin reagent, which binds to an Fc receptor.

The component which binds to an Fc receptor can also be an engineered binding protein specifically selected for binding to the Fc, and can be obtained by selection from a variegated protein display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982.

The component which binds to an Fc receptor can also be a non-immunoglobulin ligand.

The term "infectious disease" means a disorder caused by one or more species of bacteria, viruses, fungi, or protozoans, referred to as "pathogens." In this invention, pathogens are exemplified, but not limited to, *Mycobacterium tuberculosis*, *M. leprae*, *Pseudomonas aeruginosa*, *Shigella dysenteria*, *Salmonella typhi*, *S. paratyphi*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Hemophilus pneumoniae*, *Escherichia coli* serotype 0157, *Chlamydia* species, *Helicobacter* species; human immunodeficiency viruses HIV-1, -2, and -3, human herpes virus (HSV-I and -II), non-A non-B non-C hepatitis virus, human papilloma virus (HPV), cytomegalovirus (CMV), human T-cell leukemia virus (HTLV-I and II), feline leukemia virus (FeLV), simian immune deficiency virus (SIV), and rous sarcoma virus (RSV), pox viruses, rabies viruses; *Aspergillus* species; *Entamoeba histolytica*, *Giardia* species; and Newcastle disease virus.

The terms "protein," "polypeptide" and "peptide" are used interchangeably herein.

The term "substantially pure" with respect to a population of genetically modified cells means that the cells contain fewer than about 20%, more preferably fewer than about 10%, most preferably fewer than about 5%, non-modified cells.

The term "substantially pure" with respect to a nucleic acid or a protein means that the nucleic acid or protein is at least about 75%, preferably at least about 85%, more preferably at least about 90%, and most preferably at least about 95% free of other nucleic acids or proteins.

5 The term "culture medium" refers generally to any preparation suitable for cultivating living cells. A "cell culture" refers to a cell population sustained *in vitro*.

A "transgenic animal" means an animal, preferably a non-human animal having one or more cells containing a heterologous nucleic acid. The nucleic acid is introduced into the one or more cells by genetic manipulation. The term "genetic manipulation"

10 means the introduction of a recombinant DNA molecule into a cell. Typical transgenic animals of the invention express a recombinant form of a gene encoding an Fc receptor ("FcR"), such as a human FcR. A "transgenic animal" also includes an animal having an endogenous gene which is disrupted by genetic manipulation, such as a gene encoding a murine FcR.

15 The term "fusion protein" means a non-naturally occurring protein obtained from genetic manipulation of two or more genes encoding respectively two or more different proteins in the same translational reading frame (i.e., genetically linked). Translation of the fusion gene produces a fusion protein, which has elements of each of the two or more different proteins that contributed to it. Fusion proteins of the present invention

20 comprise at least a component which binds to an Fc receptor and a transmembrane protein.

The term "transmembrane protein" refers to a protein that comprises a portion that spans a biological membrane, such as a cell membrane, a nuclear membrane, or a mitochondrial membrane. The transmembrane protein portion found within the

25 membrane is the "transmembrane domain" or "transmembrane region" and is enriched in hydrophobic amino acid residues such as tryptophan, tyrosine, phenylalanine, leucine, isoleucine and valine, in comparison to, for example, extracellular or cytoplasmic domains of the protein.

The term "transgene" means a nucleic acid sequence (encoding, e.g., an FcR

30 protein), which is partly or entirely heterologous, i.e., foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its

35 insertion results in "a knockout"). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. The term "expression vector" includes any vector, (e.g., a plasmid, cosmid or phage chromosome) containing a gene construct in a form suitable for expression by a cell (e.g., linked to a promoter). In the present specification, "plasmid" and "vector" are used interchangeably, as a plasmid is a commonly used form of vector. Moreover, the invention is intended to include other vectors which serve equivalent functions.

The terms "transformation" and "transfection" mean the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell.

The term "gene product" includes an RNA molecule transcribed from a gene, or a protein translated from the gene.

Introduction

The present invention is based on the discovery that selected cells can be targeted for killing by genetically modifying the target cell (e.g. a tumor cell or a cell infected with a pathogen) to express on its surface a component which binds to an Fc receptor on an effector cell. The target cell then binds to an effector cell which processes and presents antigens associated with the target cell to immune cells, such as T and B lymphocytes, causing a cascade of immune events which result in killing (e.g., via ADCC or phagocytosis) of the target cell. Thus, the invention allows for killing of target cells without targeting any particular antigen on the cell. This provides a great advantage since many tumor cells and other target cells do not have defined (e.g., known) antigens for targeting.

In particular embodiments, the invention provides an expression vector which can be used to genetically modify (i.e., transform) a target cell to express one or more Fc receptor binding components on its surface. In another embodiments, the invention provides a target cell transformed with the aforementioned expression vector. In still other embodiments, the invention provides methods of using the expression vectors and transformed cells to effect or enhance a target cell-specific or antigen-specific immune response (e.g., to obtain a protective immune response against a particular antigen).

Expression Vectors For Genetically Modifying Target Cells

Expression vectors for use in the invention encode an anti-Fc receptor binding protein which, once expressed, is presented on the surface of a selected target cell. In all cases, the anti-Fc receptor binding protein must be sufficiently exposed on the cell surface to enable it to bind to an Fc receptor exterior to the cell.

In one embodiment, this is achieved by co-expression of the anti-Fc receptor binding component with a protein which naturally associates with (e.g., inserts into) the cell membrane (i.e., a transmembrane (TM) protein). Accordingly, expression vectors of the invention can include a chimeric gene which encodes anti-Fc receptor-TM fusion protein. Generally, the anti-Fc receptor (anti-FcR) portion of the fusion protein comprises an antibody or antibody fragment. In particular embodiments of the invention, expression vectors pJG717 encoding an H22 (anti-Fc γ R)-TM protein, and pJG718 encoding an A77 (anti-Fc α R)-TM protein are employed to transform target cells.

It will be understood that a wide range of vectors, such as described below, can be used for recombinantly expressing genes (e.g., anti-FcR antibody-transmembrane protein fusion genes) in effector cells. Such vectors can be constructed using methods well known in the art, including, without limitation, the standard techniques of restriction endonuclease digestion, ligation, plasmid and DNA and RNA purification, DNA sequencing, and the like as described, for example in Sambrook, Fritsch, and Maniatis, eds., Molecular Cloning: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. [1989]). Practitioners of ordinary skill in the art are familiar with the standard resource materials as well as specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

Any of the methods known to the art for the insertion of DNA fragments into a vector may be used to generate expression constructs of the present invention, including appropriate transcriptional/translational control signals. See, for example, Sambrook et al., *supra*; and Ausubel et al. eds. Current Protocols in Molecular Biology, (John Wiley & Sons, New York [1992]). These methods may include *in vitro* DNA recombinant and synthetic techniques and *in vivo* genetic recombination.

Anti-FcR antibody-transmembrane protein fusion genes of the present invention are typically operably linked to transcriptional regulatory sequences, such as promoters and/or enhancers, to regulate expression of the gene in a particular manner. In certain embodiments, the useful transcriptional regulatory sequences are those that are highly regulated with respect to activity, both temporally and spatially. Thus, the promoters of choice can be those that are active only in particular tissues or cell types. Where the promoter is obtained from a mammal, the mammal may be homologous (the same species as the mammal to be transformed) or non-homologous (a different species).

Appropriate promoters/enhancers can be introduced into vectors using standard methods in the art (see e.g., Maniatis). Any promoter that is sufficient to direct the initiation of transcription in a target cell may be used in the invention. For example,

promoters/enhancers which may be used to control the expression of a recombinant gene include, but are not limited to, the native transcriptional regulatory sequences for the recombinant gene (e.g., the anti-FcR antibody transmembrane fusion gene regulatory sequences or the like), the cytomegalovirus (CMV) promoter/enhancer (Keating et al. 5 (1990) *Exp Hematol* 19:99-102; and Karasuyama et al., 1989, *J Exp. Med.*, 169:13), the human β -actin promoter (Gunning et al. (1987) *PNAS* 84:4831-4835), the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al. (1984) *Mol. Cell Biol.* 4:1354-1362), the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss 10 et al. (1985) *RNA Tumor Viruses*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), the SV40 early or late region promoter (Bernoist et al. (1981) *Nature* 290:304-310; Templeton et al. (1984) *Mol. Cell Biol.*, 4:817; and Sprague et al. (1983) *J. Virol.*, 45:773), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (RSV) (Yamamoto et al., 1980, *Cell*, 22:787-797), the herpes simplex virus (HSV) 15 thymidine kinase promoter/enhancer (Wagner et al. (1981) *PNAS* 82:3567-71), and the herpes simplex virus LAT promoter (Wolfe et al. (1992) *Nature Genetics*, 1:379-384). A preferred non-tissue specific promoter is the CMV promoter (DeBernardi et al. (1991) *PNAS USA* 88:9257-61).

As an alternative to constitutive expression, the anti-FcR antibody- 20 transmembrane protein fusion gene may be placed under the control of a cell specific promoter. Examples of these promoters include globin promoters, the granzyme A promoter for expression in T-cells and NK cells, the CD34 promoter for expression in stem and progenitor cells, the CD8 promoter for expression in cytotoxic T-cells, and the CD11b promoter for expression in myeloid cells.

25 Inducible promoters may also be used for gene expression under certain physiologic conditions. Those skilled in the art will recognize a variety of inducible prokaryotic and/or eukaryotic promoters which can be used to control expression of a recombinant gene. For example, an IPTG-inducible promoter can be used to conditionally control expression of a recombinant gene. Another transcriptional control 30 system is responsive to hormones (Lee et al. (1981) *Nature* 294:228-232; Hynes et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:2038-2042; Klock et al. (1987) *Nature* 329:734-736; Israel & Kaufman (1989) *Nucl. Acids Res.* 17:2589-2604).

The vectors useful for preparing the recombinant genes of this invention typically contain one or more additional elements useful for optimizing expression in a cell. The gene construct may include transcription termination elements, such as to direct polyadenylation of an mRNA transcript, as well as intronic sequences. For example the coding sequence of the recombinant gene can be flanked at its 3' end by SV40 sequences (SV40 intron/pA) which add the transcription termination and polyadenylation signals to the transcript. In yet other embodiments, the hematopoietic gene can include intronic sequence(s) interrupting the coding sequence. In many instances, transcription of a recombinant gene in mammals is increased by the presence of one or more introns in the coding sequence.

In still other embodiments, the fusion gene construct can include additional elements which facilitate its manipulation in cells (e.g., yeast, bacterial) prior to insertion in the intended recipient cell. For instance, the vector may include origin of replication elements for amplification in prokaryotic cells. The gene construct or vector can include selectable markers for isolating transformed cells in the presence of untransformed cells, generated in a host such as a yeast or a bacterial cell used for engineering or amplifying the construct or vector. Selectable marker genes can encode proteins necessary for the survival and/or growth of transfected cells under selective culture conditions. Typical selection marker genes encode proteins that, for example: (i) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline or kanomycin for prokaryotic host cells, and neomycin, hygromycin or methotrexate for mammalian cells; or (ii) complement auxotrophic deficiencies of the cell.

The gene construct may be administered to cells (e.g., target cells to be transformed) in any biologically effective carrier, e.g. any formulation or composition capable of effectively transfecting cells *ex vivo* or *in vivo* with the expression construct. Efficient DNA transfer methods are known in the art (see, for example, Keating et al. (1990) *Exp Hematol* 18:99-102; and Dick et al. (1986) *Trends Genet* 2:165). Approaches include insertion of the gene into viral vectors including recombinant retroviruses, adenovirus and adeno-associated viruses, or recombinant bacterial or eukaryotic plasmids. Viral vectors can be used to transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (e.g. antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaPO_4 co-precipitation of recipient cells and vector. Choice of the particular gene delivery system will depend on such factors as the phenotype of the cell. Another factor in the selection of the appropriate transfection formulation is the

consideration raised by *ex vivo* transformation versus *in vivo* transformation, with the latter requiring consideration of the route of administration, e.g. locally or systemically.

One approach for either *ex vivo* or *in vivo* introduction of gene constructs of the present invention construct into cells is by use of a viral vector containing the gene as part of the virus genome carried within the virion particle. Infection of cells with a viral vector ("transduction") has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retrovirus vectors are generally understood to be one of the recombinant gene delivery system of choice for the transfer of exogenous genes into stem cells, particularly into humans cells. (see e.g., Hawley R. G., *et al* (1994) *Gene Therapy* 1: 136-38)). These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. A major prerequisite for the use of retroviruses is to ensure the safety of their use, particularly with regard to the possibility of the spread of wild-type virus in the cell population. The development of specialized cell lines (termed "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses as a gene delivery system, and defective retroviruses are well characterized for use in gene transfer for gene therapy purposes (for a review, see Miller, A.D. (1990) *Blood* 76:271). Thus, recombinant retrovirus can be constructed in which a part of the retroviral coding sequence (*gag*, *pol*, *env*) essential to viral replication has been replaced by the anti-FcR transmembrane fusion protein gene of the invention, rendering the retrovirus replication defective. The replication defective retrovirus is then packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *ex vivo* or *in vivo* with such viruses can be found in Ausubel et al., *supra*, Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include Ψ Crip, Ψ Cre, Ψ 2 and Ψ Am.

Retroviruses have been used to introduce a variety of genes into many different cell types, including embryonic stem cells, bone marrow cells, lymphocytes, hepatocytes, by both *ex vivo* and *in vivo* protocols (see for example Eglitis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA*

88:8377-8381; Chowdhury et al. (1991) *Science* 254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

Exemplary retroviral vectors have been described that yield a high titre virus capable of efficiently transducing and expressing genes in undifferentiated embryonic and hematopoietic cells (Hawley et al (1994) *Gene Therapy* 1: 136-38). These vectors contain a selectable marker (*neo*, *hph* or *pac*) under the transcriptional control of an internal murine *pgk* promoter and unique restriction sites for insertion of genes downstream of a variant LTR from the retroviral mutant PCMV (PCC4 embryonal carcinoma cell-passaged myeloproliferative sarcoma virus). A variant of the above-described retroviral vectors, the Murine Stem Cell Virus (MSCV), is illustrated in the examples set out below.

The infective spectrum of retroviruses, and consequently of retroviral-based vectors, can be limited by modifying the viral packaging proteins on the surface of the viral particle (see, for example PCT publications WO93/25234, WO94/06920, and WO94/11524). For instance, strategies for the modification of the infection spectrum of retroviral vectors include: coupling antibodies specific for stem cell surface antigens to the viral *env* protein (Roux et al. (1989) *PNAS* 86:9079-9083; Julan et al. (1992) *J. Gen Virol* 73:3251-3255; and Goud et al. (1983) *Virology* 163:251-254); or coupling cell surface ligands to the viral *env* proteins (Neda et al. (1991) *J Biol Chem* 266:14143-14146). Coupling can be in the form of the chemical cross-linking with a protein or other variety (e.g. lactose to convert the *env* protein to an asialoglycoprotein), as well as by generating fusion proteins (e.g. single-chain antibody/*env* fusion proteins). This technique, while useful to limit or otherwise direct the infection to certain tissue types, and can also be used to convert an ecotropic vector into an amphotropic vector.

To further illustrate, an anti-FcR antibody-transmembrane fusion gene construct can be generated using a retroviral vector which encodes a second fusion protein including the viral envelope protein and the vesicular stomatitis virus (VSV-G) glycoprotein (Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033-37; PCT Patent Application WO 92/14829; and WO 96/09400). Unlike typical amphotropic *env* proteins, the VSV-G protein is thought to mediate viral infection by fusing with a phospholipid component of cell membranes rather than by recognition of a cell surface protein. Since infection is not dependent on a specific receptor, VSV-G pseudotyped vectors have a broad host range. CD34⁺/Thy-1⁺ mobilized peripheral blood cells have

previously been demonstrated to be transduced with high efficiency by a VSV-G pseudotyped retroviral vector (see Kerr et al. PCT publication WO 96/09400). Genetic modification of the stem cells with a anti-FcR antibody transmembrane fusion gene construct can be accomplished at any point during their maintenance by transduction
5 with VSV-G pseudotyped virion containing the expression construct.

Moreover, use of retroviral gene delivery can be further enhanced by the use of tissue- or cell-specific transcriptional regulatory sequences which control expression of the recombinant anti-FcR antibody transmembrane fusion gene.

Another viral gene delivery system useful in the present invention utilizes
10 adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes the anti-FcR antibody transmembrane fusion gene product of interest, but is inactive in terms of its ability to replicate in a normal lytic viral life cycle (see, for example, Berkner et al. (1988) *BioTechniques* 6:616; Rosenfeld et al. (1991) *Science* 252:431-434; and Rosenfeld et al. (1992) *Cell* 68:143-155). Suitable adenoviral vectors
15 derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity with respect to stem cell populations.

20 Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up
25 to 8 kilobases) relative to other gene delivery vectors (Berkner et al., *supra*; Haj-Ahmand and Graham (1986) *J. Virol.* 57:267). Most replication-defective adenoviral vectors currently in use and therefore favored by the present invention are deleted for all or parts of the viral E1 and E3 genes but retain as much as 80% of the adenoviral genetic material (see, e.g., Jones et al. (1979) *Cell* 16:683; Berkner et al.,
30 *supra*; and Graham et al. in Methods in Molecular Biology, E.J. Murray, Ed. (Humane, Clifton, NJ, 1991) vol. 7. pp. 109-127). Expression of the inserted anti-FcR antibody transmembrane fusion gene can be under control of, for example, the E1A promoter, the major late promoter (MLP) and associated leader sequences, the E3 promoter, or exogenously added promoter sequences.

35 Yet another viral vector system useful for delivery of anti-FcR antibody-transmembrane fusion genes is the adeno-associated virus (AAV). Adeno-associated viral vectors have been shown to be effective at transducing other genes into pluripotent

anti-FcR antibody transmembrane fusion stem cells *in vitro* (see PCT Application WO 96/08560). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al. *Curr. Topics in Micro and Immunol.* (1992) 158:97-129). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al. (1992) *Am. J. Respir. Cell. Mol. Biol.* 7:349-356; Samulski et al. (1989) *J. Virol.* 63:3822-3828; and McLaughlin et al. (1989) *J. Virol.* 62:1963-1973). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al. (1985) *Mol. Cell. Biol.* 5:3251-3260 can be used to a recombinant anti-FcR antibody transmembrane fusion gene into stem cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470; Tratschin et al. (1985) *Mol. Cell. Biol.* 4:2072-2081, Wondisford et al. (1988) *Mol. Endocrinol.* 2:32-39, Tratschin et al. (1984) *J. Virol.* 51:611-619; and Flotte et al. (1993) *J. Biol. Chem.* 268:3781-3790).

Anti-Fc Receptor Binding Components

As previously described, anti-Fc receptor binding components of the present invention include antibodies, functional antibody fragments (i.e., which retain binding capacity or the ability to recognize a particular epitope), peptides which mimic antibody binding and other Fc receptor binding agents. In a preferred embodiment, the Fc receptor binding component is expressed as a fusion protein which, when expressed by a target cell, comprises a portion which is exposed on the surface of the target cell and a portion which is inserted in the membrane of the cell (i.e., a transmembrane portion which favors a lipid environment). When expressed in this manner, the Fc receptor binding component is anchored to the target cell via the transmembrane portion. In one embodiment, the fusion protein comprises an antibody portion and a transmembrane domain portion. In particular embodiments, the antibody portion is derived from monoclonal antibodies referred to as H22 (anti-FcγR) and A77 (anti-FcαR) described herein.

The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active determinants of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as an Fab or single chain Fv fragment. Nucleic acids encoding anti-FcR antibodies suitable for use in the present invention (i.e., which can be used to transform target cells)

are available in the art (e.g., from the American Type Culture Collection, Rockville, MD, or commercially, e.g., from Becton-Dickinson or Immunotech), or can be prepared by standard techniques. Accordingly, the following is an overview of such techniques. For purposes of clarity, most of these techniques are referred to in terms of preparing antibodies and antibody fragments, instead of the preparing nucleic acids (e.g., genes) encoding antibodies and antibody fragments. However, it is known to those of ordinary skill in the art that once an antibody with a set of desired (e.g., anti-FcR) binding characteristics is prepared, the antibody can be sequenced and/or cloned using art recognized techniques (e.g., PCR) to provide a nucleic acid encoding the antibody.

Structurally, the simplest naturally occurring antibody (e.g., IgG) comprises four polypeptide chains, two copies of a heavy (H) chain and two of a light (L) chain, all covalently linked by disulfide bonds. Specificity of binding in the large and diverse set of antibodies is found in the variable (V) determinant of the H and L chains; regions of the molecules that are primarily structural are constant (C) in this set.

The binding sites of the proteins that comprise an antibody, i.e., the antigen-binding functions of the antibody, are localized by analysis of fragments of a naturally-occurring antibody. Thus, antigen-binding fragments are also intended to be designated by the term "antibody." Examples of binding fragments encompassed within the term antibody include: a Fab fragment consisting of the V_L , V_H , C_L and C_{H1} domains; an Fd fragment consisting of the V_H and C_{H1} domains; an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; a dAb fragment (Ward et al., 1989 *Nature* 341:544-546) consisting of a V_H domain; an isolated complementarity determining region (CDR); and an $F(ab')_2$ fragment, a bivalent fragment comprising two Fab' fragments linked by a disulfide bridge at the hinge region. These antibody fragments are obtained using conventional techniques well-known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

An anti-FcR "antibody" of the invention can further include a bispecific molecule or chimeric molecule having at least one antigen binding determinant derived from an anti-FcR antibody, or a single chain anti-FcR antibody. Although the H and L chains are encoded by separate genes, a synthetic linker can be made that enables these chains to be made as a single protein chain (known as single chain antibody, sc-Ab), or to be recombinantly expressed as a single protein chain (Bird et al. 1988 *Science* 242:423-426; and Huston et al. 1988 *PNAS* 85:5879-5883).

The term "monoclonal antibody" or "monoclonal antibody composition" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a

particular epitope. Monoclonal antibodies can be prepared using a technique which provides for the production of antibody molecules by continuous growth of cells in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, *Nature* 256:495-497; see also Brown et al.

5 1981 *J. Immunol* 127:539-46; Brown et al., 1980, *J Biol Chem* 255:4980-83; Yeh et al., 1976, *PNAS* 76:2927-31; and Yeh et al., 1982, *Int. J. Cancer* 29:269-75) and the more recent human B cell hybridoma technique (Kozbor et al., 1983, *Immunol Today* 4:72), EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96), and trioma techniques.

10 A monoclonal antibody can be produced by the following steps. In all procedures, an animal is immunized with an antigen such as a protein (or peptide thereof) as described above for preparation of a polyclonal antibody. The immunization is typically accomplished by administering the immunogen to an immunologically competent mammal in an immunologically effective amount, i.e., an amount sufficient
15 to produce an immune response. Preferably, the mammal is a rodent such as a rabbit, rat or mouse. The mammal is then maintained on a booster schedule for a time period sufficient for the mammal to generate high affinity antibody molecules as described. A suspension of antibody-producing cells is removed from each immunized mammal secreting the desired antibody. After a sufficient time to generate high affinity
20 antibodies, the animal (e.g., mouse) is sacrificed and antibody-producing lymphocytes are obtained from one or more of the lymph nodes, spleens and peripheral blood. Spleen cells are preferred, and can be mechanically separated into individual cells in a physiological medium using methods well known to one of skill in the art. The antibody-producing cells are immortalized by fusion to cells of a mouse myeloma line.
25 Mouse lymphocytes give a high percentage of stable fusions with mouse homologous myelomas, however rat, rabbit and frog somatic cells can also be used. Spleen cells of the desired antibody-producing animals are immortalized by fusing with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol. Any of a number of myeloma cell lines suitable as a fusion partner are used with to standard
30 techniques, for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines, available from the American Type Culture Collection (ATCC), Rockville, Md.

The desired hybridomas obtained from among the fused cells are cultured in selective medium such as HAT medium, designed to eliminate unfused parental
35 myeloma or lymphocyte or spleen cells. Hybridoma cells are selected and are grown under limiting dilution conditions to obtain isolated clones. The supernatants of each clonal hybridoma is screened for production of antibody of desired specificity and

affinity, e.g., by immunoassay techniques to determine the desired antigen such as that used for immunization. Monoclonal antibody is isolated from cultures of producing cells by conventional methods, such as ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography (Zola *et al.*, Monoclonal Hybridoma
5 Antibodies: Techniques And Applications, Hurell (ed.), pp. 51-52, CRC Press, 1982). Hybridomas produced according to these methods can be propagated in culture *in vitro* or *in vivo* (in ascites fluid) using techniques well known to those with skill in the art.

For therapeutic use of antibodies of non-human origin in humans, the non-human "foreign" epitopes elicit immune response in the patient. If sufficiently developed, a
10 potentially lethal disease known as HAMA (human antibodies against mouse antibody) may result. To eliminate or minimize HAMA, it is desirable to engineer chimeric antibody derivatives, i.e., "humanized" antibody molecules that combine the non-human Fab variable region binding determinants with a human constant region (Fc). Such antibodies are characterized by equivalent antigen specificity and affinity of monoclonal
15 and polyclonal antibodies described above, and are less immunogenic when administered to humans, and therefore more likely to be tolerated by the patient.

Chimeric mouse-human monoclonal antibodies (i.e., chimeric antibodies) can be produced by recombinant DNA techniques known in the art. For example, a gene encoding the Fc constant region of a murine (or other species) monoclonal antibody
20 molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. (see Robinson *et al.*, International Patent Publication PCT/US86/02269; Akira, *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger
25 *et al.*, International Application WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988 *Science* 240:1041-1043); Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.*, 1987, *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.*, 1987, *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.*, 1988, *J. Natl*
30 *Cancer Inst.* 80:1553-1559.)

The chimeric antibody can be further humanized by replacing sequences of the Fv variable region which are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General reviews of humanized chimeric antibodies are provided by Morrison, S. L., 1985, *Science* 229:1202-1207 and by Oi *et al.*, 1986, *BioTechniques* 4:214. Those methods include isolating, manipulating, and
35 expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Sources of such nucleic acid

are well known to those skilled in the art and, for example, may be obtained from 7E3, an anti-GPIIb/IIIa antibody producing hybridoma. The recombinant DNA encoding the chimeric antibody, or fragment thereof, can then be cloned into an appropriate expression vector. Suitable humanized antibodies can alternatively be produced by CDR substitution (U.S. Patent 5,225,539; Jones et al. 1986 *Nature* 321:552-525; Verhoeyan et al. 1988 *Science* 239:1534; and Beidler et al. 1988 *J. Immunol.* 141:4053-4060).

All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to the Fc receptor.

An antibody can be humanized by any method, which is capable of replacing at least a portion of a CDR of a human antibody with a CDR derived from a non-human antibody. Winter describes a method which may be used to prepare the humanized antibodies of the present invention (UK Patent Application GB 2188638A, filed on March 26, 1987), the contents of which is expressly incorporated by reference. The human CDRs may be replaced with non-human CDRs using oligonucleotide site-directed mutagenesis as described in International Application WO 94/10332 entitled, *Humanized Antibodies to Fc Receptors for Immunoglobulin G on Human Mononuclear Phagocytes*.

Also within the scope of the invention are chimeric and humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, preferred humanized antibodies have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, in a humanized antibody having mouse CDRs, amino acids located in the human framework region can be replaced with the amino acids located at the corresponding positions in the mouse antibody. Such substitutions are known to improve binding of humanized antibodies to the antigen in some instances. Antibodies in which amino acids have been added, deleted, or substituted are referred to herein as modified antibodies or altered antibodies.

The term modified antibody is also intended to include antibodies, such as monoclonal antibodies, chimeric antibodies, and humanized antibodies which have been modified by, e.g., deleting, adding, or substituting portions of the antibody. For example, an antibody can be modified by deleting the constant region and replacing it with a constant region meant to increase half-life, e.g., serum half-life, stability or affinity of the antibody. Any modification is within the scope of the invention so long as the bispecific and multispecific molecule has at least one antigen binding region specific for an FcγR and triggers at least one effector function.

Human monoclonal antibodies (HumAb antibodies) directed against human proteins can be generated using transgenic mice carrying the complete human immune system rather than the mouse system. Splenocytes from these transgenic mice immunized with the antigen of interest are used to produce hybridomas that secrete human mAbs with specific affinities for epitopes from a human protein (see, e.g., Wood et al. International Application WO 91/00906, Kucherlapati et al. PCT publication WO 91/10741; Lonberg et al. International Application WO 92/03918; Kay et al. International Application 92/03917; Lonberg, N. et al. 1994 *Nature* 368:856-859; Green, L.L. et al. 1994 *Nature Genet.* 7:13-21; Morrison, S.L. et al. 1994 *Proc. Natl. Acad. Sci. USA* 81:6851-6855; Bruggeman et al. 1993 *Year Immunol* 7:33-40; Tuailon et al. 1993 *PNAS* 90:3720-3724; Bruggeman et al. 1991 *Eur J Immunol* 21:1323-1326).

Monoclonal antibodies can also be generated by other methods known to those skilled in the art of recombinant DNA technology. An alternative method, referred to as the "combinatorial antibody display" method, has been developed to identify and isolate antibody fragments having a particular antigen specificity, and can be utilized to produce monoclonal antibodies (for descriptions of combinatorial antibody display see e.g., Sastry et al. 1989 *PNAS* 86:5728; Huse et al. 1989 *Science* 246:1275; and Orlandi et al. 1989 *PNAS* 86:3833). After immunizing an animal with an immunogen as described above, the antibody repertoire of the resulting B-cell pool is cloned. Methods are generally known for obtaining the DNA sequence of the variable regions of a diverse population of immunoglobulin molecules by using a mixture of oligomer primers and PCR. For instance, mixed oligonucleotide primers corresponding to the 5' leader (signal peptide) sequences and/or framework 1 (FR1) sequences, as well as primer to a conserved 3' constant region primer can be used for PCR amplification of the heavy and light chain variable regions from a number of murine antibodies (Larrick et al., 1991, *Biotechniques* 11:152-156). A similar strategy can also be used to amplify human heavy and light chain variable regions from human antibodies (Larrick et al., 1991, *Methods: Companion to Methods in Enzymology* 2:106-110).

In an illustrative embodiment, RNA is isolated from B lymphocytes, for example, peripheral blood cells, bone marrow, or spleen preparations, using standard protocols (e.g., U.S. Patent No. 4,683,202; Orlandi, et al. *PNAS* (1989) 86:3833-3837; Sastry et al., *PNAS* (1989) 86:5728-5732; and Huse et al. (1989) *Science* 246:1275-1281.) First-strand cDNA is synthesized using primers specific for the constant region of the heavy chain(s) and each of the κ and λ light chains, as well as primers for the signal sequence. Using variable region PCR primers, the variable regions of both heavy and light chains are amplified, each alone or in combination, and ligated into appropriate vectors for further manipulation in generating the display packages.

Oligonucleotide primers useful in amplification protocols may be unique or degenerate or incorporate inosine at degenerate positions. Restriction endonuclease recognition sequences may also be incorporated into the primers to allow for the cloning of the amplified fragment into a vector in a predetermined reading frame for expression.

5 The V-gene library cloned from the immunization-derived antibody repertoire can be expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. Ideally, the display package comprises a system that allows the sampling of very large variegated antibody display
10 libraries, rapid sorting after each affinity separation round, and easy isolation of the antibody gene from purified display packages. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia *Recombinant Phage Antibody System*, catalog no. 27-9400-01; and the Stratagene *SurfZAP*TM phage display kit, catalog no. 240612), examples of methods and reagents particularly amenable for
15 use in generating a variegated antibody display library can be found in, for example, Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No.
20 WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J* 12:725-734; Hawkins et al. (1992) *J Mol Biol* 226:889-896; Clackson et al. (1991) *Nature* 352:624-628; Gram et al. (1992) *PNAS* 89:3576-3580; Garrad et al. (1991) *Bio/Technology* 9:1373-1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133-4137; and Barbas et al. (1991) *PNAS* 88:7978-7982.

 In certain embodiments, the V region domains of heavy and light chains can be expressed on the same polypeptide, joined by a flexible linker to form a single-chain Fv fragment, and the scFV gene subsequently cloned into the desired expression vector or
30 phage genome. As generally described in McCafferty et al., *Nature* (1990) 348:552-554, complete V_H and V_L domains of an antibody, joined by a flexible (Gly₄-Ser)₃ linker can be used to produce a single chain antibody which can render the display package separable based on antigen affinity. Isolated scFv antibodies immunoreactive with the antigen can subsequently be formulated into a pharmaceutical preparation for use in the
35 subject method.

Once displayed on the surface of a display package (e.g., filamentous phage), the antibody library is screened with the Fc γ R, or peptide fragment thereof, to identify and isolate packages that express an antibody having specificity for the Fc γ R. Nucleic acid encoding the selected antibody can be recovered from the display package (e.g., from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques.

Immune response to "foreign" antigens comprises the notion that "self" proteins and other molecules expressed within an organism are not antigenic or immunogenic to that organism. In fact, discrimination between isologous or homologous determinants and "foreign," or heterologous determinants is achieved through maturation of the immune system of an organism during development of the immune system. A system of selection against immune cells bearing antibodies with binding determinants to "self" occurs, so that when mature the immune system does not attack proteins or other molecules native to the organism. In certain pathological conditions known as "autoimmune diseases," however, such discrimination is not as accurate, and endogenous structures may be subject to attack from the immune system. Examples of autoimmune diseases and conditions in which there is autoimmune exacerbation of symptoms include systemic lupus erythematosus, myasthenia gravis, multiple sclerosis, and rheumatoid arthritis. One autoimmune disease, idiopathic thrombocytopenic purpura (ITP), which affects approximately 150,000 patients in the United States (according to the U.S. Department of HHS, 1992 data), results from macrophages in the spleen and liver removing autoantibody-coated platelets from circulation. The autoantibodies generally have the IgG isotype (Schwarz, R.S., Autoimmunity and Autoimmune Diseases, 1993, p. 1075. In: Fundamental Immunology, 3rd Ed., W.E. Paul, Ed., Raven Press, NY), and the disease results in continuous platelet destruction.

Other Fc receptor binding components include peptides which mimic the binding of anti-Fc receptor antibodies of the invention, such as antibodies H22 and A77 described herein. Such "peptide mimetics" can be designed and generated by those of ordinary skill in the art according to known techniques, such as those described by Saragovi et al. (1991) *Science* 253:792; Hinds et al. (1991) *J. Med. Chem.* 34(6):1777; Jenks et al. (1992) *J. Natl Cancer Inst.* 84:79; and Fassine et al. (1994) *Immunomethods* 5:121.

Fc Receptors

Fc receptors are divided among four classes known as Fc γ receptor, Fc α receptor, Fc μ receptor, Fc ϵ receptor) which bind to IgG, IgA, IgM, IgE, respectively.

Receptors for IgG molecules, in particular, are known as Fc γ R, of which Fc γ RI is
5 a high affinity receptor found on dendritic cells, monocytes and macrophages, and are
inducible on neutrophils and eosinophils (Van de Winkel JGI, *et al.*, 1993, *Immunol Today* 14:215). The lower affinity IgG receptors are Fc γ RII, found on neutrophils
(polymorphonuclear neutrophils, PMNs), monocytes, and platelets, and Fc γ RIII, found
10 on macrophages, PMNs, and natural killer cells (NKs). The low affinity IgG receptors
are also found on mast cells and subsets of T cells (Ravetch *et al.*, 1991, *Ann. Rev. Imm.* 9:457). Biological functions associated with binding of IgG to these receptors include
phagocytosis, superoxide generation, cytotoxicity, and triggering mediator release. The
biological role of Fc γ RI has not been fully determined, since because of its high affinity
it might be saturated with IgG *in vivo*, suggesting a steady state situation. Data
15 including induction of expression of Fc γ RI during streptococcal infection (Guyre, P.M *et al.*, 1990, *J. Clin. Invest.* 86:1892), induction during IFN- γ treatment of patients with
chronic granulomatous disease, and on neutrophils from a negligible quantity in healthy
individuals to a large number during acute inflammation (Davis, BH *et al.*, *supra*)
suggest an important role in resistance to infection (Van de Winkel JGI, *et al.*, 1993,
20 *Immunol Today* 14; 215).

Effector Cells and Antigen Presentation

As used herein, the term "effector cell" refers to an immune cell which is
involved in the effector phase of an immune response, as opposed to the cognitive and
25 activation phases of an immune response. Exemplary immune cells include a cell of a
myeloid or lymphoid origin, e.g., lymphocytes (e.g., B cells and T cells including
cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes,
eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and
basophils. Effector cells express specific Fc receptors and carry out specific immune
30 functions. In preferred embodiments, an effector cell is capable of inducing antibody-
dependent cellular toxicity (ADCC), e.g., a neutrophil capable of inducing ADCC. For
example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which
express Fc α R are involved in specific killing of target cells and presenting antigens to
other components of the immune system, or binding to cells that present antigens. In
35 other embodiments, an effector cell can phagocytose a target antigen, target cell, or
microorganism. The expression of a particular FcR on an effector cell can be regulated

by humoral factors such as cytokines. For example, expression of Fc γ RI has been found to be up-regulated by interferon gamma (IFN- γ). This enhanced expression increases the cytotoxic activity of Fc γ RI-bearing cells against targets. An effector cell can phagocytose a target antigen or a target cell. An effector cell can also lyse a target cell.

5 An "effector cell specific antibody" as used herein refers to an antibody or functional antibody fragment that binds the Fc receptor of effector cells. Preferred antibodies for use in the subject invention bind the Fc receptor of effector cells at a site which is not bound by endogenous immunoglobulin.

10 Using the methods and compositions provided herein, effector cells are targeted to cells associated with (e.g., which naturally or recombinantly express) a target antigen. For example, effector cells can be directed via their Fc receptors to a tumor cells, such as TAG-72-bearing cells. Exemplary TAG-72-bearing cells include carcinoma or adenocarcinoma-derived cells (e.g., colon, breast, prostate, ovarian and endometrial cancer cells) (Thor, A. *et al.* (1997) *Cancer Res* 46: 3118; Soisson A. P. *et al.* (1989) *Am. J. Obstet. Gynecol.*:1258-63). Alternatively, effector cells can be directed via their
15 Fc receptors to cells infected with a pathogen, such as HIV-1.

20 The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, ovarian carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes
25 carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term
"sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

30 Antigen uptake through antigen-antibody complexes bound to Fc receptors for IgG (Fc γ R) increases the efficiency of antigen presentation and thereby antigen-specific T-cell activation by human and mouse macrophages, (Celis et al (1984) *Science* 224:297-299; Chang (1985) *Immunol. Today* 6:245-259; Manca et al. (1988) *Immunol.* 140:2893-2898; Schalke et al. (1985) *J. Immunol.* 134:3643-3648; and Snider et al (1987) *J. Immunol.* 139:1609-1616). The binding of these complexes to Fc γ R is mediated by the Fc region of the antibody. This binding is susceptible to inhibition by physiological concentration of IgG.

35 An optimal antibody response to a thymus-dependent antigen requires that the B cell obtain help from CD4+ helper T cell. The B cell is uniquely designed to accomplish this in that it contains antigen-specific immunoglobulin on its surface which allows it to

bind, internalize and process antigen for presentation very efficiently. Other antigen presenting cells, such as the macrophage and dendritic cell, lack antigen-specific receptors, and therefore also lack this highly efficient mechanism for processing and presenting antigen. However, the apparent requirement for adjuvants when

5 administering vaccines suggests a need for an antigen presenting cell in addition to the B cell. Also, it appears that antigen presentation by resting B cells to resting T cells does not lead to a T cell activation, but rather to T cell tolerance (Eynon et al. (1992) J. Esp. Med. 175:131). This is due to the failure of the resting B cell to deliver all the signals required for activation of the resting T cell. On the other hand, it appears that induction
10 of T cell tolerance by the resting B cell could be averted if the resting T cell first responds to antigen on the antigen presenting cell such as the macrophage or dendritic cell (Parker et al. (1991) FASEB J. 5:2777). This implies that in the naive individual, the resting T cell must first interact with a macrophage or dendritic cell before interacting with the resting B cell.

15 These considerations have lead to the conclusion that the optimal immunogen requires two major components: antigen which can be recognized by the antigen-specific B cell; and a component which directs antigen for efficient processing and presentation to an antigen presenting cell other than the resting B cell (Parker et al., *ibid.*; Germain (1991) Nature 353:605). Attaching antigens to anti-Fc receptor antibodies satisfies these
20 criteria since antigen directed to Fc receptors on the macrophage enhances antigen presentation at least 100 fold (Immunol. Today (1985) 6:245). Studies performed in vivo support the efficacy of such a vaccine. For example, a substantial increase in antibody production has been observed following immunization of mice with bispecific antibody which directed antigen to MHC class II or Fc γ RII (Snider et al. (1990) (J. Exp.
25 Med. 171:1957-1963). In addition, the requirement for adjuvant was eliminated. The ability to use substantially lower doses of immunogens is especially valuable when administering immunogens such as allergens that are potentially toxic at higher doses. Tolerance against some allergens can be obtained by repeated low dose administration of the allergen.

30 To construct an immunogen for human use which would satisfy the above criteria, the observation that antigen-antibody complexes can significantly enhance antigen presentation was expanded. When antigen-antibody complexes bind to an FcR (e.g., Fc γ R or Fc α R)
on the monocyte or macrophage, the antigen is internalized and its subsequent
35 presentation and thus T cell activation, is dramatically enhanced *in vitro* (Chang (1985) Immunol. Today 6:245), decreasing the antigen concentration required for T cell activation by 10 to 100-fold. The data presented here demonstrate the potential for using

FcR-targeted immunogens and target cells as vaccines and to enhance antigen presentation.

In the methods of this invention, antigens expressed within or on the surface of a target cell can be targeted to an antigen-presenting cell to enhance the processes of internalization and presentation by these cells, and ultimately, to stimulate an immune response therein.

Preferred surface receptors of antigen-presenting cells for targeting are the receptors for the Fc region of IgG (FcγR). These receptors mediate internalization of antibody-complexed antigens. The Fcγ receptors include FcγRI, FcγRII, and FcγRIII. The most preferred target is the high affinity Fc receptor (FcγRI).

Other preferred Fc receptors which may be targeted include Fcα receptors. Binding of ligand to FcαR triggers phagocytosis and antibody mediated cell cytotoxicity in leukocytes and FcαR-bearing cell lines. Fcα receptors can also cooperate with receptors for IgG on effector cells in enhancing the phagocytosis of target cells. Monoclonal antibodies of the IgM (Shen, L. *et al.*, 1989 *J. Immunol.* 143: 4117) and IgG (Monteiro, R.C. *et al.*, 1992 *J. Immunol.* 148: 1764) classes have been developed against FcαR.

The production and characterization of monoclonal antibodies which bind FcγRI without being blocked by human IgG are described by Fanger et al. in PCT application WO 88/00052 and in U.S. Patent No. 4,954,617, and in U.S. Patent No. 5,635,600, the teachings each of which are incorporated by reference herein. These antibodies bind to an epitope of FcγRI which is distinct from the Fc binding site of the receptor and, thus, their binding is not blocked substantially by physiological levels of IgG. Specific anti-FcγRI antibodies useful in this invention are mAb 22, mAb 32, mAb 32.2, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32.2 (an identical sub-clone of mAb 32) is available from the American Type Culture Collection, Rockville, MD, ATCC No. HB9469.

Antibody preparations suitable for directing the cell or virus displaying an anti-FcαR binding determinant to an effector all displaying FcαR have been described (Monteiro et al. 1992, *J. Immunol.* 148:1764-1770; and U.S.S.N. 08/678,194, which is hereby incorporated by reference). Monteiro et al. describe mAb A77 and A3, which specifically bind FcαR at an epitope such that binding is not inhibited by human IgA.

Pharmaceutical Compositions

The Examples below are not intended as delimiting with respect to the nature of the therapeutic agent such as a vector or a genetically modified cell, or to a particular route of the administration and additional routes are listed herein, *infra*.

In another embodiment of the present invention, the compositions of the invention (e.g., expression vectors and transformed (targeted) cells) can be administered by combination therapy, i.e., combined with other agents. For example, the combination therapy can include a composition of the present invention with at least one other vector or a genetically modified cell, at least one antibiotic, or other conventional therapy.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The use of such media and agents for pharmaceutically active substances is well known in the art. Preferably, the carrier is suitable for oral, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound can be coated in a material to protect the compound from the action of acids and other natural conditions that can inactivate the compound.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than oral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

When administered *in vivo*, transformed cells and expression vectors of the present invention can be administered (e.g., as a pharmaceutical composition) to subjects using a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response, such as protective immunity against tumor cells or a pathogen). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

One of ordinary skill in the art can determine and prescribe the effective amount of the pharmaceutical composition required. For example, one could start doses at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the invention will be that amount of the composition which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will

generally depend upon the factors described above. It is preferred that administration be intravenous, intramuscular, intraperitoneal, or subcutaneous.

Uses and Methods

5 The compositions and methods of the present invention can be used to achieve targeted immunostimulation (e.g., against target antigens or target cells) either *in vitro* or *in vivo*.

 For *in vitro* use, immunocompetent effector cells are separated and purified from patient blood. These effector cells are then contacted in culture with the antigen in
10 association with a cell or virus displaying the FcR binding determinant. The antigen-presenting effector cells will process the antigen and present fragments on their surface. When immune cells, such as T and B lymphocytes are present, an antigen-specific immune response can be stimulated.

 Alternatively, target cells associated with an antigen can be transformed to
15 express an FcR binding component *ex vivo* and then returned to patients to elicit a specific effector cell-mediated immune response via the above-summarized process. The cells are administered in a pharmacologically acceptable solution at a dosage which will evoke an immune response against the antigen. The cells may be irradiated prior to administration to remove the possibility of the transformed cells growing inside the
20 patient. Further, the patient's cells (e.g. blood cells) may be removed, fractionated and cultured if appropriate to expand the cell number, treated *ex vivo* and returned to the patient for therapy. Further, *ex vivo* cultured cells may be treated at various points during *ex vivo* culture and expansion, with agents to modify expression or activity of certain functional anti-FcR binding components. Agents include but are not limited to, growth
25 factors, cytokines, lymphokines such as IFN- γ , G-CSF, TNF, and GM-CSF, and interleukins such as IL-2, IL-10 and IL-12.

 The optimum dose of cells, as well as the molar ratio of antigen and binding agent, may vary dependent upon factors such as the type of antigen, the immune status of the host, the type of tumor or infection or other disease being treated, etc. In most
30 cases, the does of cells required to elicit an immune response (as determined by any standard method for assessment of immune response) should be lower than that which would be required if the antigen were given alone or as a complex with a monospecific antibody for the antigen (Snider et al., *ibid.*).

 The methods provided by the present invention can be used to enhance or
35 reinforce the immune response to an antigen or to multiple antigens simultaneously. For example, the methods can be used to treat chronic infections, such as hepatitis and AIDS, where the unaided immune systems is unable to overcome the infection. The

methods can also be used to treat acute stages of infection when reinforcement of an immune response against the invading organism may be necessary.

The methods can further be used to reduce the dose of antigen required to obtain a protective or therapeutic immune response or in instances when the host does not respond or responds minimally to the antigen. Although generally desirable, the lowering of effective dose can be especially desirable when the antigen is toxic to the host such as is the case for allergies.

The methods can also be used in disease therapy for cancer. For example, transformed cells of the invention can be used to target tumor-associated (or tumor-specific) antigens or tumor cells to antigen-presenting cells in order to cause or to enhance an immune response against the tumor. Importantly, the tumor antigen(s) need not be known, as the whole tumor cell is targeted to the effector cell. Indeed, many tumor cells express several different tumor-specific antigens. In a preferred embodiment, transformed target cells of the invention are cancer cells, particularly cancer cells from breast, ovary, testis, lung, colon, rectum, pancreas, liver, central nervous system, head and neck, kidney, bone, blood and lymphatic system cancers. Suitable target antigens among such cancer cells include members of the human EGF-like receptor family, such as EGF receptor, the cancer cell antigens HER-2/*neu*, HER-3, HER-4, and a heteromultimeric receptor comprised of at least one HER subunit. Additional cancer cell antigens include carcinoembryonic antigen, gastrin releasing peptide receptor antigen, and TAG 72. TAG 72 has been identified on about 90% of colorectal cancers, 85% of breast tumors, and 95% of ovarian tumors (Johnson et al.(1986) *Cancer Res.* 46:850-897; Bodmer, M. *et al.*, European Patent Specification 0 348 442 B1; Mezes, P. *et al.* International Application WO 93/12231).

In another embodiment, transformed cells of the invention target infectious disease antigens to effector cells. Infectious disease antigens include those from bacteria, fungi, protozoa, and viruses, such as HIV, HTLV and FELV, protozoan (such as *Toxoplasma gondii*), fungal (such as *Candida albicans*); and bacterial (such as *Staphylococcus aureus*, *Streptococcus hemolyticus* and *Mycobacterium tuberculosis*).

Another type of antigen which can be targeted by way of the present invention is an allergen. An "allergen" refers to a substance that can induce an allergic or asthmatic response in a susceptible subject. The number of allergens that elicit a sensitive response in a proportion of a population is enormous, and includes pollens, insect venoms, animal dander, dust mite proteins, fungal spores and drugs (e.g. penicillin). Examples of natural animal and plant allergens include proteins specific to the following genera: *Felis* (*Felis domesticus*); *Canis* (*Canis familiaris*); *Dermatophagoides* (e.g. *Dermatophagoides farinae*); *Periplaneta* (e.g. *Periplaneta americana*); *Ambrosia* (*Ambrosia artemisiifolia*;

Lolium (e.g. *Lolium perenne* or *Lolium multiflorum*); *Cryptomeria* (*Cryptomeria japonica*); *Alternaria* (*Alternaria alternata*); *Alder*; *Alnus* (*Alnus gultinosa*); *Betula* (*Betula verrucosa*); *Quercus* (*Quercus alba*); *Olea* (*Olea europa*); *Artemisia* (*Artemisia vulgaris*); *Plantago* (e.g. *Plantago lanceolata*); *Parietaria* (e.g. *Parietaria officinalis* or *Parietaria judaica*); *Blattella* (e.g. *Blattella germanica*); *Apis* (e.g. *Apis multiflorum*); *Cupressus* (e.g. *Cupressus sempervirens*, *Cupressus arizonica* and *Cupressus macrocarpa*); *Juniperus* (e.g. *Juniperus sabinoideis*, *Juniperus virginiana*, *Juniperus communis* and *Juniperus ashei*); *Thuya* (e.g. *Thuya orientalis*); *Chamaecyparis* (e.g. *Chamaecyparis obtusa*); *Agropyron* (e.g. *Agropyron repens*); *Secale* (e.g. *Secale cereale*); *Triticum* (e.g. *Triticum aestivum*); *Dactylis* (e.g. *Dactylis glomerata*); *Festuca* (e.g. *Festuca elatior*); *Poa* (e.g. *Poa pratensis* or *Poa compressa*); *Avena* (e.g. *Avena sativa*); *Holcus* (e.g. *Holcus lanatus*); *Anthoxanthum* (e.g. *Anthoxanthum odoratum*); *Arrhenatherum* (e.g. *Arrhenatherum elatius*); *Agrostis* (e.g. *Agrostis alba*); *Phleum* (e.g. *Phleum pratense*); *Phalaris* (e.g. *Phalaris arundinacea*); *Paspalum* (e.g. *Paspalum notatum*); *Sorghum* (e.g. *Sorghum halepensis*); and *Bromus* (e.g. *Bromus inermis*).

Many allergens are found in airborne pollens of ragweed, grasses, or trees, or in fungi, animals, house dust, or foods. As a class, they are relatively resistant to proteolytic digestion. Preferable allergens are those which bind to IgE on mast cells and basophils, thereby causing a range of symptoms from inflammation and asthma to a type I anaphylaxis hypersensitivity reaction.

The methods of the present invention can also be used to vaccinate against diseases and cancers by including by causing "redirected" targeted immunostimulation against antigens associated with such diseases and cancers. Breast and ovarian cancers are sex hormone dependent cancers. Breast tumors may be characterized by abnormally expressed receptors, e.g. those of the human-EGF-like receptor family (HER), for example HER-2, -3, and 4. The invention is not limited to these embodiments of HER antigens. The natural HER ligand, heregulin, can be incorporated into a bispecific antibody (BsAb) or multispecific molecule, as a means to target a breast tumor cell expressing one or more HER receptor during cancer. Further, heregulin molecules are binding determinants for heterodimeric HER receptors containing, eg. a monomer of each of HER-2, -3 or -4 in combination.

Additional examples of sex hormone-dependent cancer include prostate cancer (Smith, P. (1995), *Cancer Surveys Vol. 23: Preventing Prostate Cancer*, Imper. Cancer Research Fund and testicular cancers). The growth of hormone-dependent cancer types is promoted by male hormones (e.g., androgens such as testosterone and dihydrotestosterone). Removal of the testes (castration) was for many years the standard method of preventing secretion of male hormones by the gonads, to reduce growth of the

cancer. Currently, secretion of male hormones is suppressed by chemical means by interfering with production of luteinizing hormone (LH), which regulates synthesis of male hormones. Similar considerations are applicable to other sex hormone-dependent cancers, such as breast or ovarian cancer, so that patients with these diseases or in a population prone to these diseases, are usually not administered sex hormones as therapeutic or replacements.

In one embodiment, the nestin protein for brain cancers and which is expressed during normal mammalian fetal development, and is also expressed on tumors of the central nervous system, including most forms of brain cancer (McKay, D.G. Ronald, *U.S. Patent No. 5,338,839*, 8/16/94) is used as a target antigen. Nestin is also expressed on melanomas on the skin and on melanomas that have metastasized (V.A. Florenes, R. Holm, O. Myklebost, U. Lendahl, O. Fodstad, *Cancer Res.* 54: 354-6, 1994), to other organs and are difficult to detect and treat. The preferred site of delivery for treatment of a brain tumor with the molecules of this invention is directly into the central nervous system or directly, to the brain via spinal injection or fine needle delivery. For a metastatic cancer, a preferred delivery route would be by direct injection into the circulation, or by the *ex vivo* blood methods described herein.

Other tumor types include Wilm's tumor (A.J. Buckler, K.M. Call, T.M. Glaser, D.A. Haber, D.E. Housman, C.Y. Ito, J. Pelletier, Rose, E.A. Rose, *U.S. Patent No. 5,350,840*) a pediatric kidney cancer due to occurrence of a somatic mutation in the patient's single copy of a gene normally found in two intact copies. Wilm's tumor can be cured surgically in 95% of cases, and a bispecific or multispecific multivalent binding protein is envisioned to be suitable as an adjunct therapeutic modality for surgical patients. Other examples of known cancer-associated proteins for which the compositions of matter and methods of the current invention are suitable include those associated with gastrointestinal cancer (R. Fishel *et al.*, International Application WO 95/14085, 05/26/95), those characterized by development of multiple drug resistance during chemotherapy (J.M. Croop *et al.*, *U.S. Patent No. 5,198,344*), and a large number of oncogenes well known to the skilled artisan such as *Rb*, *ras*, and *c-myc*, the sequences of which are available for analysis to those with skill in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those herein can be used in the practice or testing of the present invention, the preferred methods and materials are described below.

The invention is further illustrated by the following examples, which should not be construed as further limiting. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. The contents of all references, pending patent applications and published patents, cited throughout this application, are hereby
5 expressly incorporated by reference.

EXAMPLES

Materials and Methods

10 The following methodology described in the Materials and Methods section was used throughout the Examples, set forth below.

Cell Lines and Monoclonal Antibodies

The anti-Fc γ receptor antibody is a humanized form of monoclonal antibody 22
15 (H22), described in U.S.P.N. 5,635,600, which is incorporated by reference. The production and characterization of the H22 antibody is described in Graziano, R.F. et al. (1995) *J. Immunol* 155 (10): 4996-5002 and PCT/US93/10384. The H22 antibody producing cell line was deposited at the American Type Culture Collection on November 4, 1992 under the designation HA022CL1 and has the ATCC accession
20 number CRL 11,177.

Other specific anti-Fc γ RI antibodies useful in this invention are mAb 32.2, mAb 44, mAb 62 and mAb 197. The hybridoma producing mAb 32.2 is available from the American Type Culture Collection, ATCC accession number HB9469.

The murine hybridoma producing cell line for the anti-Fc α R antibody is
25 exemplified by A77 (Monteiro *et al.* 1992, *J. Immunol.* 148: 1764-1770).

The anti-FcR mAbs were purified from each respective hybridoma supernatant by protein A affinity chromatography (Bio-Rad, Richmond, CA).

Preparation of Blood Cells

30 Leukocytes were prepared from heparinized whole venous blood or from apheresis of normal human volunteers. Whole blood was diluted with RPMI containing 5% dextran at a ratio of 2.5:1 (v/v). The erythrocytes were allowed to sediment for 45 minutes on ice, then the cells in the supernatant were transferred to a new tube and pelleted by centrifugation. The residual erythrocytes were removed by hypotonic lysis.
35 The remaining lymphocytes, monocytes and neutrophils were kept on ice until use in binding assays. For some experiments, neutrophils were separated from mononuclear cells by ficoll hypaque (Pharmacia-Upjohn, Piscataway, NJ) gradient separation.

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Monocytes were enriched from mononuclear cells by cold aggregation and settling through a cushion of fetal calf serum. Monocyte cultures were used fresh or were incubated at 37°C, 5% CO₂ for 24 to 48 hours in teflon dishes at 4 x 10⁶ cells/ml of MSFM containing 2.0% normal human serum type AB (Sigma, St. Louis, MO) and 500 IRU/ml IFN-γ (R&D Systems, Minneapolis, MN). Neutrophils were cultured for 24 to 48 hours (37°C, 5% CO₂) in AIM V media (Gibco/BRL, Grand Island, NY) with 50 ng/ml G-CSF (Kindly provided by R. Repp, U. of Erlanger, Germany) and 500 IRU/ml IFN-γ.

10 ***Binding by flow cytometry***

The binding of anti-idiotypic antibody (22 ID) or soluble FcγRI (ligand) to H22-TM displayed on transformed cells was assessed by flow cytometry. Various concentrations of fluorescent antibody or sFc diluted in PBS, pH 7.4 containing 2mg/ml BSA and 0.05% NaN₃ (PBA), were incubated with transformed NSO cells or with transformed MTC cells for one hour at 0°C. The cells were washed with PBA and incubated with a phycoerythrin labeled goat anti-mouse antibody for one hour at 0°C. The cells were washed and fixed with 1% paraformaldehyde, and cell associated fluorescence was analyzed on a Becton Dickinson (Mountain View, CA) FACScan.

20 **Example 1 - preparation of a plasmid expression vector for surface expression of single chain antibody specific for FcγR**

A plasmid expression vector (Figure 1) to direct surface expression of the single chain antibody portion (sFv) of anti- FcγRI mAb H22 was engineered by inserting a DNA fragment encoding the H22 sFv in frame with the sequence for the transmembrane domain of platelet derived growth factor receptor (PDGF-R) using a commercial plasmid vector pDisplay (Invitrogen, Inc., 1600 Faraday Ave, Carlsbad, CA 92008; Cat. No. V660-20\$210).

pDisplay is a 5,325 base pair mammalian expression vector that is designed to target recombinant proteins to the surface of mammalian cells via the transmembrane domain of PDGF-R. An H22 sFv fragment protein was targeted and anchored to the surface of recipient cells (NSO and MTC cells) by cloning a DNA encoding the H22 sFv fragment in frame with the N-terminal cell surface targeting signal and the C-terminal transmembrane anchoring domain of PDGF-R, encoding fusion protein H22-TM

The pDisplay plasmid features: a CMV promoter (bases 1-596); a T7 promoter/priming site for *in vitro* transcription of sense RNA and for sequencing of inserts (bases 638-657); the murine Igk-chain V-J2-C signal peptide (bases 737-799); hemagglutinin A epitope (bases 800-826); multiple cloning site including sites SfiI,

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BglI, XmaI, SacII, PstI, SalI and AccI (bases 827-873); *myc* epitope (bases 874-903); PDGF-R transmembrane domain (bases 907-1,056); bovine growth hormone polyadenylation signal (bases 1069-1288); ColE1 origin for growth in *Escherichia coli* (bases 1378-2051); thymidine kinase polyadenylation site (bases 2458-2187); neomycin resistance marker for stable selection in mammalian cells (bases 3421-2366, transcribed in counterclockwise direction); SV40 origin and promoter, for replication and simple vector rescue in cell lines expressing the large T-antigen (bases 3797-3456, counterclockwise); ampicillin-resistance gene for selection for selection in *E. coli* (4736-3876); and fl origin (5000-5099).

10 Plasmid pDisplay was digested with BglIII and SacII, and then ligated to DNA encoding H22 sc-Fv consisting of VL-linker VH similarly digested. The plasmid pJG717 was obtained from ampicillin-resistant colonies obtained from transformed *E. coli*, and its structure was verified by appropriate restriction digests.

15 **Example 2 - Surface Expression of H22 in Murine Tumor Cells**

Murine tumor cell lines, 653 and NSO (ATCC), were transformed with the H22-TM vector pJG717 (SEQ ID NO:1), and each cell line was established to have stable expression of the H22-TM fusion protein on cell surfaces as confirmed by flow cytometry using a soluble rabbit anti-H22 antibody, and with a soluble form of FcγRI (see Figure 2).

20 Untransformed 653 and NSO cells were used as controls. Cells were incubated with soluble rabbit anti-H22 antibody for 60 min. at 4°C. After washing the cells, antibody bound to cells was detected by a donkey anti-rabbit IgG-phycoerytherin probe. The transformed cells showed a significant shift in fluorescence when incubated with anti-H22 antibodies. These transformed cells were specifically lysed with G-CSF/IFN-γ treated granulocytes. Only the transformed cells were lysed by the effector cells and only the H22 F(ab')₂ fragments were able to inhibit the lysis of the transformed cells.

25 These results demonstrate that tumor cells can be transformed to express an anti-FcγR binding component, such as a single chain fragment of H22, on the surface of the cell (e.g., as a transmembrane fusion protein), thereby enabling the tumor cell to bind to soluble FcγRI.

30 **Example 3 - Surface Expression of H22 sFv Engages Effector Cells and Triggers FcγRI Effector Functions**

The following studies were performed to demonstrate that expression of H22-TM by tumor cells can lead to the engagement of FcγRI on effector cells, such as monocytes and activated granulocytes, thereby initiating FcγRI-dependent effector functions, such as cell lysis (e.g., ADCC), phagocytosis and cytokine secretion.

ADCC of H22-TM Transformed Cells by Activated Granulocytes

Chromium-labeled target murine tumor cells (NSO and 653) were transformed with pJG717 encoding the H22-TM fusion as described in Example 1 to act as target cells for cytokine activated granulocytes. These transformed cell lines were designated as NSO-22TM and 553-22TM, respectively. ADCC was measured using a chromium release assay.

In particular, granulocytes were purified from normal whole blood and cultured overnight with G-CSF and IFN- γ . The effector cells were combined with ^{51}Cr -labeled untransfected myeloma cells, 653-22TM cells, or NSO-22TM cells at an effector to target ratio of 50:1. H22 F(ab')₂ or A77F(ab')₂ was added in excess to demonstrate specific blocking of the ADCC. Cytotoxicity was calculated by the formula: % lysis = (experimental CPM - target leak CPM/detergent lysis CPM - target leak CPM) X 100%. Specific lysis was measured by % lysis with antibody - % lysis without antibody. Assays were performed in triplicate. The results for the NSO-22TM cells are shown in Figure 3. The results for the 653-22TM cells are shown in Figure 5.

As shown in Figures 3 and 5, only those cells which expressed the H22-TM were killed by the granulocytes. Further, this activity was substantially or completely blocked by addition of soluble H22 F(ab')₂ antibody fragments. Untransformed control cells that did not express H22-TM were not lysed.

These data demonstrate that anti-Fc γ RI antibody determinants (e.g., sFv H22) can be expressed on the surface of tumor cells so the cells, when in the presence of activated granulocytes, engage and activate Fc γ RI resulting in specific lysis of the tumor cells. Furthermore, the lysis of 653-H22-TM cells could be specifically inhibited by addition of excess H22 F(ab')₂ fragments that bind to Fc γ RI, but not by A77F(ab')₂ fragments that bind to Fc α R.

ADCC of H22-TM Transformed Cells by Monocytes

Chromium-labeled target murine myeloma 653 cells were transformed with pJG717 encoding the H22-TM fusion as described in Example 1 to act as target cells for macrophages. As in the previously described experiments, ADCC was measured using a chromium release assay.

In particular, monocytes were purified from normal human donors and differentiated into macrophages in the presence of IFN- γ . The effector cells were combined with ^{51}Cr -labelled untransfected myeloma cells or ^{51}Cr -labelled 653-22TM cells at an effector to target ratio of 30:1 and incubated for 3.5 hours. H22 F(ab')₂ or A77F(ab')₂ was added in excess to demonstrate specific blocking of the ADCC.

As shown in Figure 6, when 653-22TM verses untransformed 653 myeloma cells were incubated alone with effector cells, only the 653-22TM cells were killed by the granulocytes. Further, this activity was substantially blocked by addition of soluble H22 F(ab')₂ antibody fragments.

5 These data demonstrate that anti-FcγRI antibody determinants (e.g., sFv H22) can be expressed on the surface of tumor cells so the cells, when in the presence of macrophages, engage and activate FcγRI resulting in specific lysis of the tumor cells. Furthermore, the lysis of 653-H22-TM cells could be specifically inhibited by addition of excess H22 F(ab')₂ fragments that bind to FcγRI, but not by A77F(ab')₂ fragments that
10 bind to FcαR.

Phagocytosis of H22-TM Transformed Cells

A murine carcinoma cell line (MTC) was transformed with the H22-TM vector pJG717, and cell lines were established that have stable expression of the fusion protein
15 on their plasma membrane as described in Example 1.

The functional expression of the H22-TM was demonstrated by enhanced phagocytosis of the two H22-TM expressing cells liens (MTC-22-A1, and MTC-22-A4) as compared to non-transformed MTC cells. In particular, phagocytosis was evaluated by a two-color flow cytometry assay, in which the target cells were labeled with a red
20 fluorescent dye, and the effector cells were labeled with a green fluorescent dye. Thus, when macrophage engulfed the target cells, the macrophages had both green and red fluorescence.

Briefly, macrophages were cultured from monocytes in media containing M-CSF and IFN-γ. MTC cells were labeled with PKH-26 dye (Sigma) and cultured with
25 macrophages in 24 well plated for 18 hours. The macrophages were stained with CD14-FITC before analysis of samples with a FACScan. The % phagocytosis was determined by the formula: (number of dual positive cells) / (total number of red cells) x 100%. The effector to target ratio was 20:1. The results are shown in Figure 7 which shows a significantly higher percentage of phagocytosis for transformed tumor cells
30 (MTC-22-A1 and MTC-22-A4) compared to untransformed tumor cells (MTC). This demonstrates that transformation of tumor cells to express H22-TM substantially increases phagocytosis of the tumor cells by effector cells.

Induction of Cytokines by H22-TM Transformed Cells

35 A murine myeloma cell line (653) was transformed with the H22-TM vector pJG717, and cells lines were established that have stable expression of the fusion protein on their plasma membrane as described in Example 1. The functional expression of the

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H22-TM was demonstrated by eliciting the secretion of specific cytokine from effector cells as compared to non-transformed 653 cells. For example, the 653-22TM cell showed a specific induction of IL-1 β , TNF- α , and IL-6 cytokines.

5 In particular, monocytes were purified from normal whole blood and allowed to adhere to plastic tissue culture plates. The monocytes were con-cultured with 653 cells or 653-22TM cells at an effector to target ratio of 5:1. Media was removed from the cultures at 0, 4, and 21 hours and measured for the presence of cytokines by commercially available kits.

10 The results are shown in Figure 8 which shows that 653-22TM cells were able to specifically induce IL-1 β , TNF- α , and IL-6 cytokines. This demonstrates that transformation of tumor cells to express H22-TM causes the specific induction of cytokines.

15 **Example 4 - Targeting antigen presenting cells to tumors expressing antibody for Fc γ R**

Transgenic mice expressing Fc γ RI can be used to evaluate the anti-tumor and antigen presentation capacity of the H22-TM vector. Tumor cells that express selected tumor-specific antigens can be transformed with vectors such as pJG717, and expression of the H22-TM will efficiently target these cells to antigen presenting cells (e.g., dendritic cells, monocytes and macrophages).

20 The antigen presenting cells can then phagocytose the H22-bearing cells through binding of Fc γ RI, and initiate an immune reaction specific to the antigens present, by expressing peptides derived from proteolytic cleavage of the tumor antigen, displayed on MHC molecules. In this manner, specific immunity against cells bearing such tumor antigens can be achieved. This can be done *in vivo* in patients or, alternatively in transgenic animals, such as transgenic mice expressing human Fc γ RI.

25 **Example 5 - Irradiation of genetically modified cells ex vivo prior to administration to a subject**

30 Cells expressing a tumor or a pathogen antigen can be engineered *ex vivo* to express H22-TM or a similar construct that binds an FcR, and can then be administered to a subject to generate a specific immune response. For this application the engineered cells that are "redirected" to bind an FcR can be irradiated prior to administration, to reduce or eliminate growth of the transformed cells in the patient (e.g., to ensure less than about 10% survival of the irradiated cells).

35

Example 6 - Surface Expression of A77 sFv on Murine Myeloma Cells

Murine myeloma cells (NSO) were transformed with an expression vector referred to as pJG718 (Figure 9) encoding a fusion protein similar H22-TM as described in Example 1, except that the single chain anti-Fc γ RI portion (sFv H22) was replaced by a single chain anti-Fc α R portion from antibody A77 (sFv A77). This fusion protein is referred to as A77-TM.

Cell lines were established that have stable expression of the fusion protein on their plasma membrane. As with H22-TM in Example 2, functional expression of the A77-TM in NSO cells was demonstrated by flow cytometry. In particular, the NSO A77-TM transformed cell line (77-D6) was incubated with soluble Fc α R (5 μ g/ml) for 90 min. at 4°C. After washing the cells, IgA was added (20 μ g/ml) was added for 90 min. at 4°C. Again, the cells were washed, and the IgA bound to cells was detected by a goat anti-human IgA-phycoerytherin probe.

The results are shown in Figure 11 which shows that transformed NSO A77-TM cells exhibited a significant shift in fluorescence, but only when both soluble Fc α R and IgA were added. Panel A shows that cells reacted only with probe, Panel B shows that cells reacted with soluble Fc α R and probe, Panel C shows that cells reacted with IgA and probe, Panel D shows that cells reacted with soluble Fc α R and IgA and probe. Overall, these results demonstrate that tumor cells transformed to express A77-TM can bind a soluble form of the Fc α R, and the bound Fc α R can still engage IgA molecules.

EQUIVALENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

All patents and publications referred to herein are hereby incorporated by reference.

What is claimed is:

1. A cell transformed to express on its surface a component which binds to an Fc receptor of an effector cell.
- 5 2. The cell of claim 1, wherein the component is an antibody or an antigen binding fragment thereof.
3. The cell of claim 2, wherein the fragment is a single chain Fv fragment.
- 10 4. The cell of claim 2, wherein the antibody is selected from the group consisting of an IgA, an IgG and fragments thereof.
5. The cell of claim 1, wherein the component which binds to the Fc receptor is produced recombinantly in the cell.
- 15 6. The cell of claim 4, wherein binding of the antibody to the Fc receptor is not blocked by IgA or IgG.
7. The cell of claim 5 wherein the component binds to an Fc γ receptor or an Fc α receptor.
- 20 8. The cell of claim 1 which is a mammalian cell.
9. The cell of claim 1 further comprising on its surface an antigen selected from the group consisting of a tumor antigen and a component of a pathogen.
- 25 10. The cell of claim 1 which is a tumor cell.
11. The cell of claim 9, wherein the tumor antigen is selected from the group consisting of HER-2/*neu*, TAG 72, carcinoembryonic antigen, and gastrin releasing peptide. The cell of claim 5, wherein the tumor expresses an antigen selected
- 30 12. The cell of claim 9, wherein the pathogen is a virus.
- 35 13. The cell of claim 9, wherein the pathogen is a bacterium or a fungus.

- 40 -

14. The cell of claim 1, which is transformed *ex vivo* to express the component which binds to the Fc receptor.
15. The cell of claim 1, wherein the Fc receptor is selected from the group consisting of an Fc γ receptor, an Fc α receptor, an Fc μ receptor, and an Fc ϵ receptor.
16. The cell of claim 1, wherein the Fc receptor is selected from the group consisting, of Fc γ I, Fc γ II, and Fc γ III.
17. The cell of claim 1, wherein the component which binds to the Fc receptor is expressed recombinantly as a fusion protein comprising a transmembrane protein and a Fc receptor binding protein.
18. The cell of claim 17, wherein the transmembrane protein comprises the transmembrane domain of a platelet derived growth factor receptor.
19. The cell of claim 2, wherein the antibody is selected from the group consisting of antibody H22 having ATCC deposit number CRL 11,177, and antibody A77.
20. The cell of claim 19, wherein the fragment is a single chain Fv fragment of antibody H22 or A77.
21. The cell of claim 17, wherein the fusion protein comprises a single chain Fv fragment of antibody H22 or antibody A77 and a transmembrane protein.
22. A cell transformed to express a fusion protein comprising (a) an antibody or antibody fragment which binds to an Fc receptor of an effector cell, and (b) a transmembrane protein.
23. The cell of claim 22, wherein the antibody fragment is a single chain Fv fragment of antibody H22 having ATCC deposit number CRL 11,177 or of antibody A77.
24. The cell of claim 22, wherein the transmembrane protein is the transmembrane domain of a platelet derived growth factor receptor.
25. The cell of claim 22, which is a tumor cell transformed *ex vivo* to express the fusion protein.

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26. The cell of claim 22, which is a cell infected with a pathogen, and wherein the cell is transformed *ex vivo* to express the fusion protein.
- 5 27. A method of increasing an immune response in a subject comprising administering to the subject a cell transformed to express on its surface a component which binds to an Fc receptor of an effector cell.
- 10 28. The method of claim 27 further comprising administering to the subject an agent that increases expression of Fc receptors on effector cells.
29. The method of claim 28, wherein the agent is a cytokine.
- 15 30. The method of claim 29, wherein the cytokine is selected from the group consisting of G-CSF, GM-CSF, IFN- γ , TNF, and combinations thereof.
31. The method of claim 27, wherein the cell is a tumor cell.
- 20 32. The method of claim 27, wherein the cell is transformed *ex vivo*, and then administered to the subject.
- 25 33. A method of increasing an immune response to an antigen, comprising transforming a cell which expresses the antigen with a nucleic acid encoding a protein which binds to an Fc receptor on an effector cell; and contacting the cell with an effector cell in the presence of a lymphocyte.
34. The method of claim 33 wherein transforming the cell is performed *ex vivo*, and contacting the cell with an effector cell is performed *in vivo*.
- 30 35. The method of claim 35, wherein the nucleic acid encodes an antibody or antigen binding fragment thereof.
- 35 36. The method of claim 33, wherein the antibody comprises antibody H22 having ATCC number CRL 11,177, or antibody A77.
37. The method of claim 36, wherein the antibody fragment comprises a single chain Fv fragment of H22 or A77.

38. The method of claim 33, wherein the nucleic acid encodes a fusion protein comprising an antibody or antibody fragment and a transmembrane protein.
- 5 39. The method of claim 33, wherein the antigen is selected from the group consisting of a tumor antigen and a component of a pathogen.
40. An expression vector encoding a fusion protein comprising a portion which binds to an Fc receptor on an effector cell and a transmembrane protein.
- 10 41. The expression vector cell of claim 40, wherein the transmembrane protein comprises the transmembrane domain of a platelet derived growth factor receptor.
42. The expression vector cell of claim 40, wherein the portion that binds to an Fc receptor comprises an antibody or an antigen binding fragment thereof.
- 15 43. The expression vector cell of claim 42, where the antibody is selected from the group consisting of humanized antibody H22 having ATCC deposit number CRL 11,177, and antibody A77.
- 20 44. The expression vector cell of claim 42, wherein the antigen binding fragment is a single chain Fv fragment which binds to an Fc γ receptor or an Fc α receptor.
45. The expression vector cell of claim 40, wherein the Fc receptor is an Fc γ receptor or an Fc α receptor.
- 25

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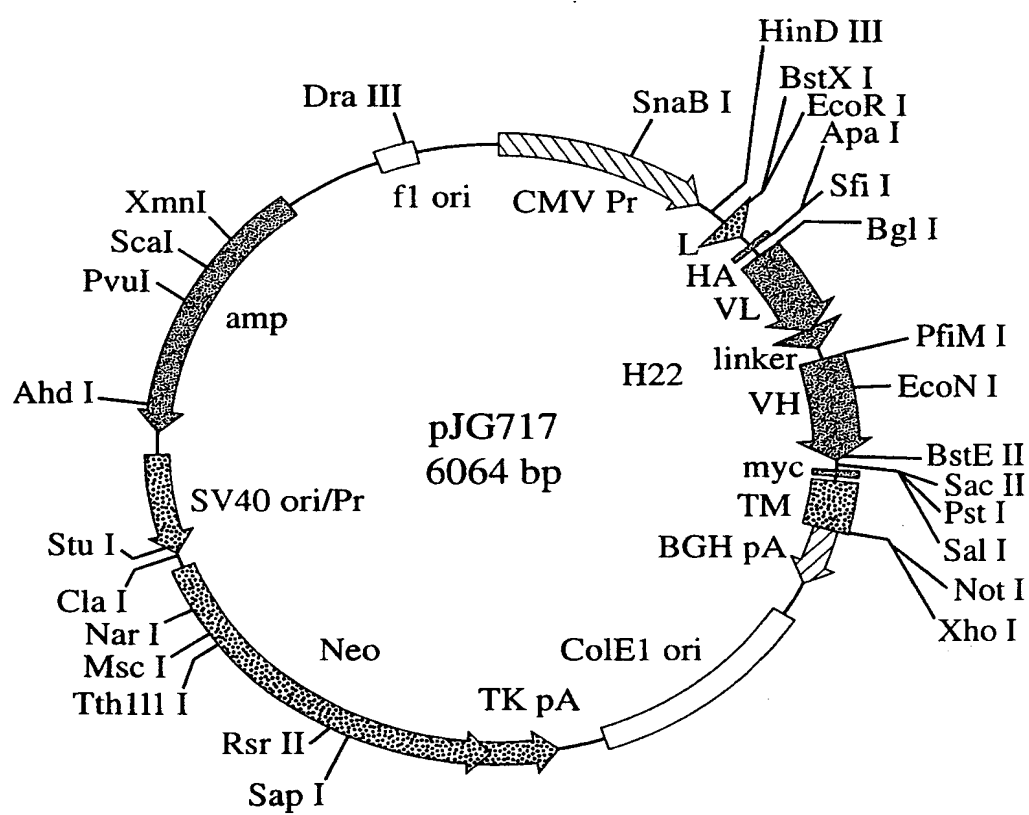
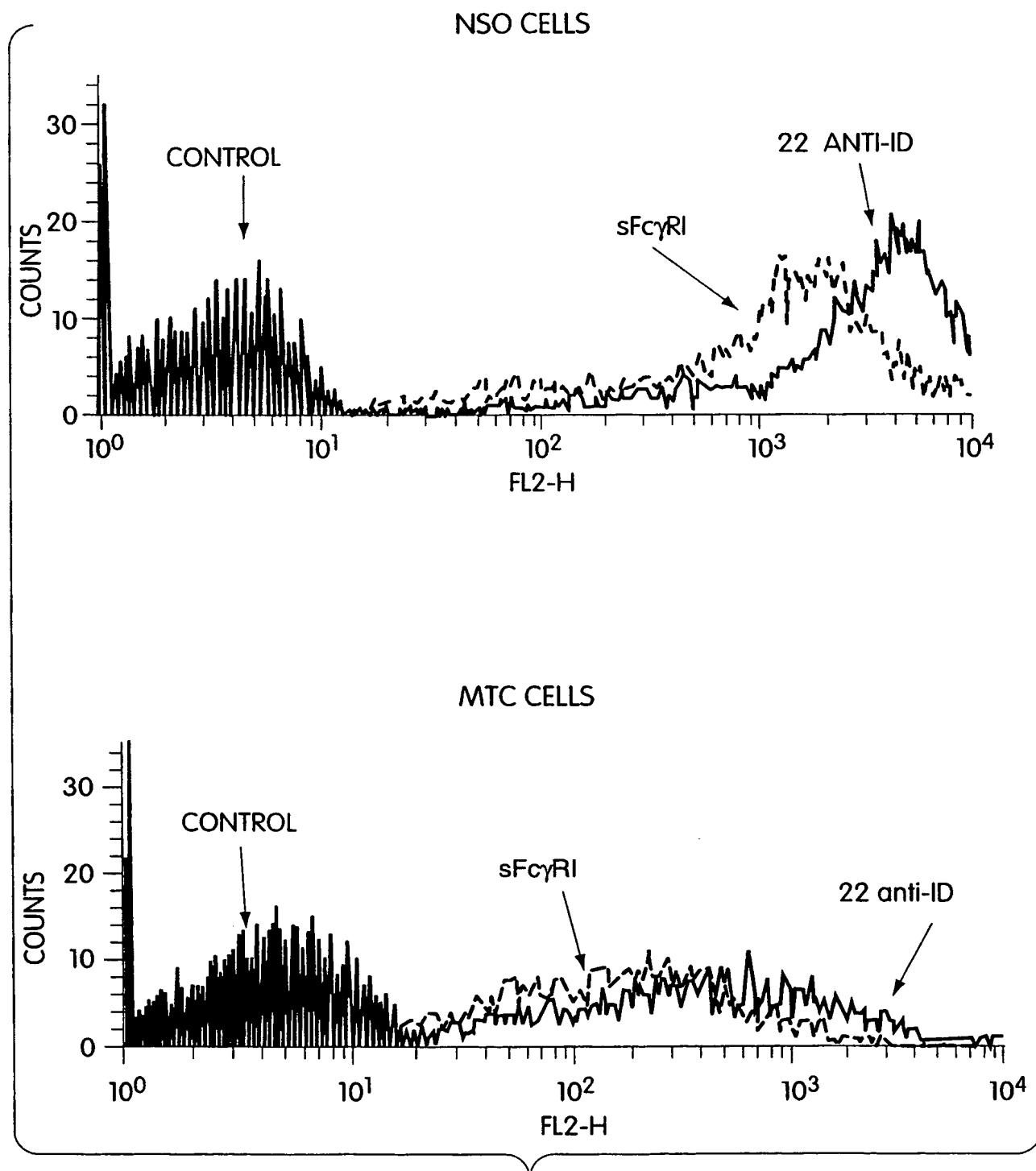


Fig. 1

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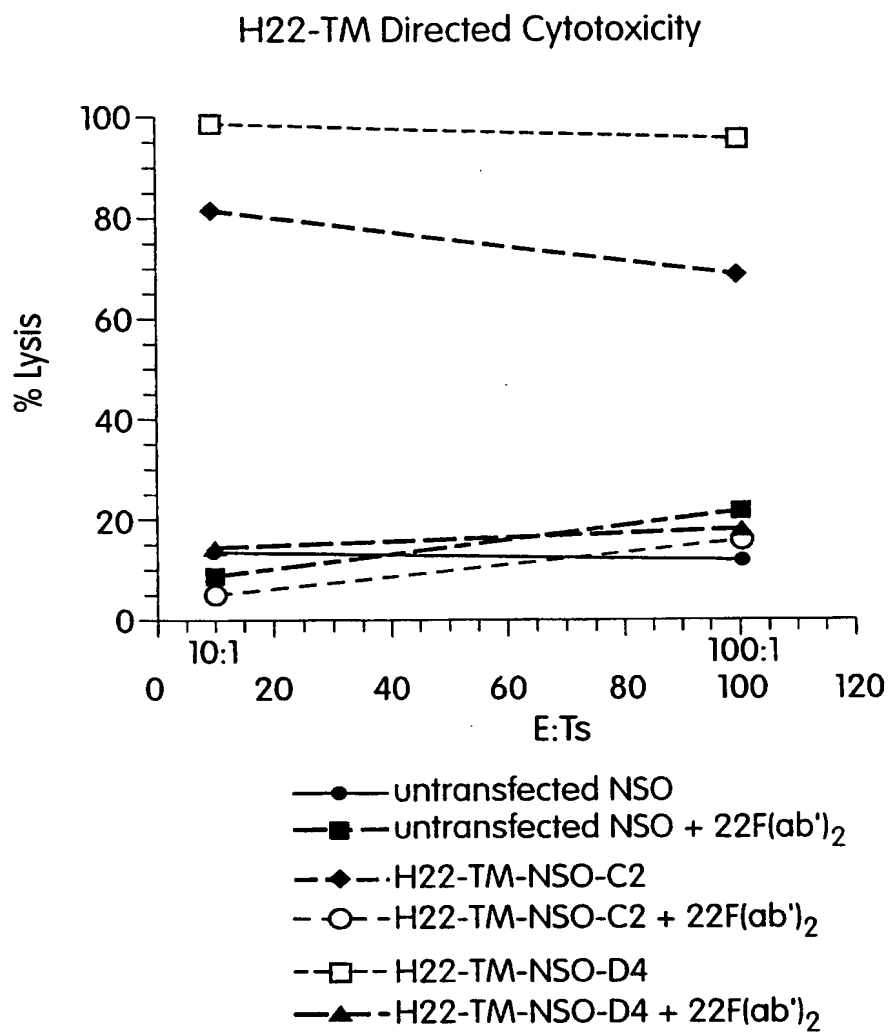


Fig. 3

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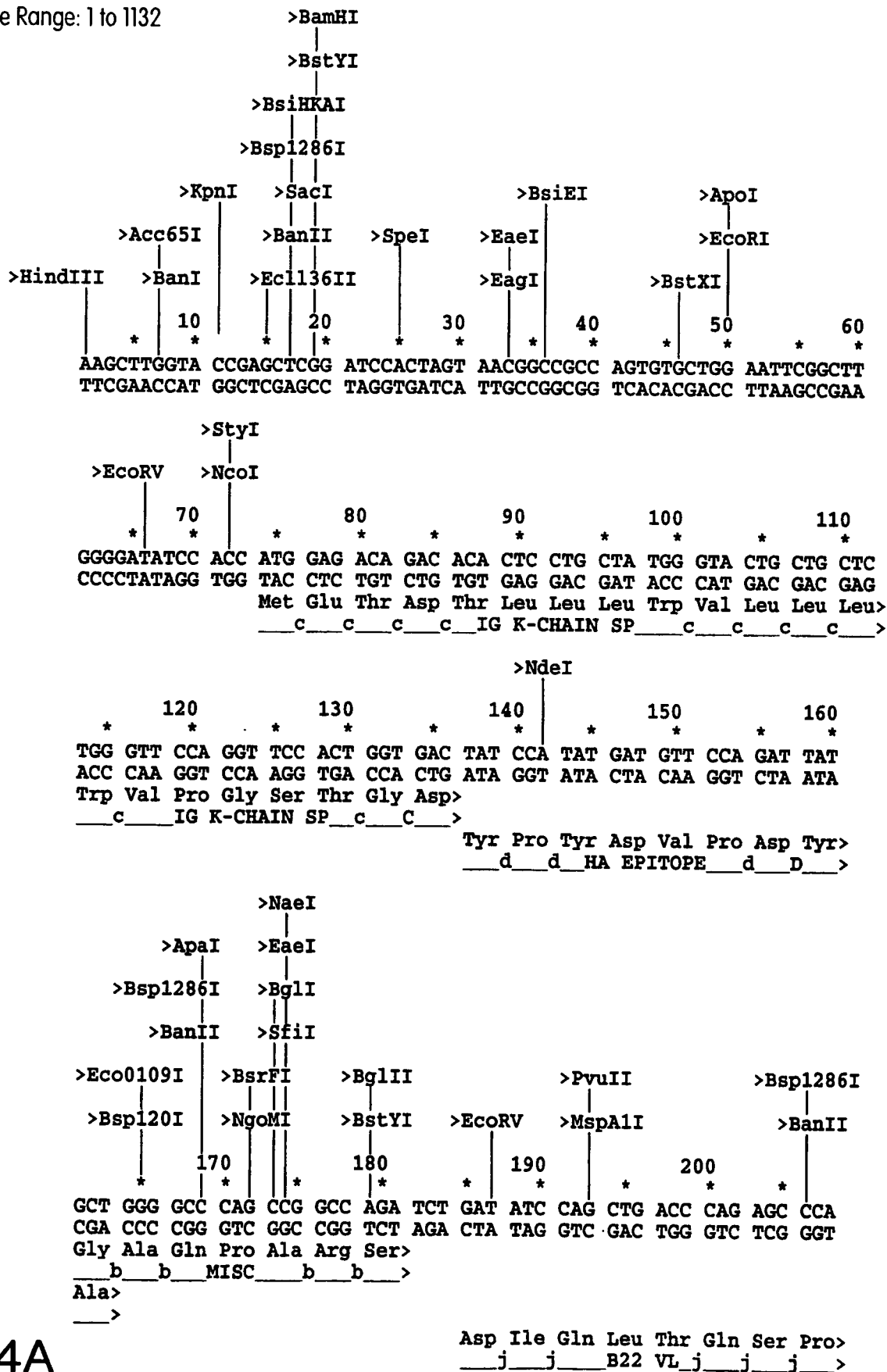


Fig. 4A

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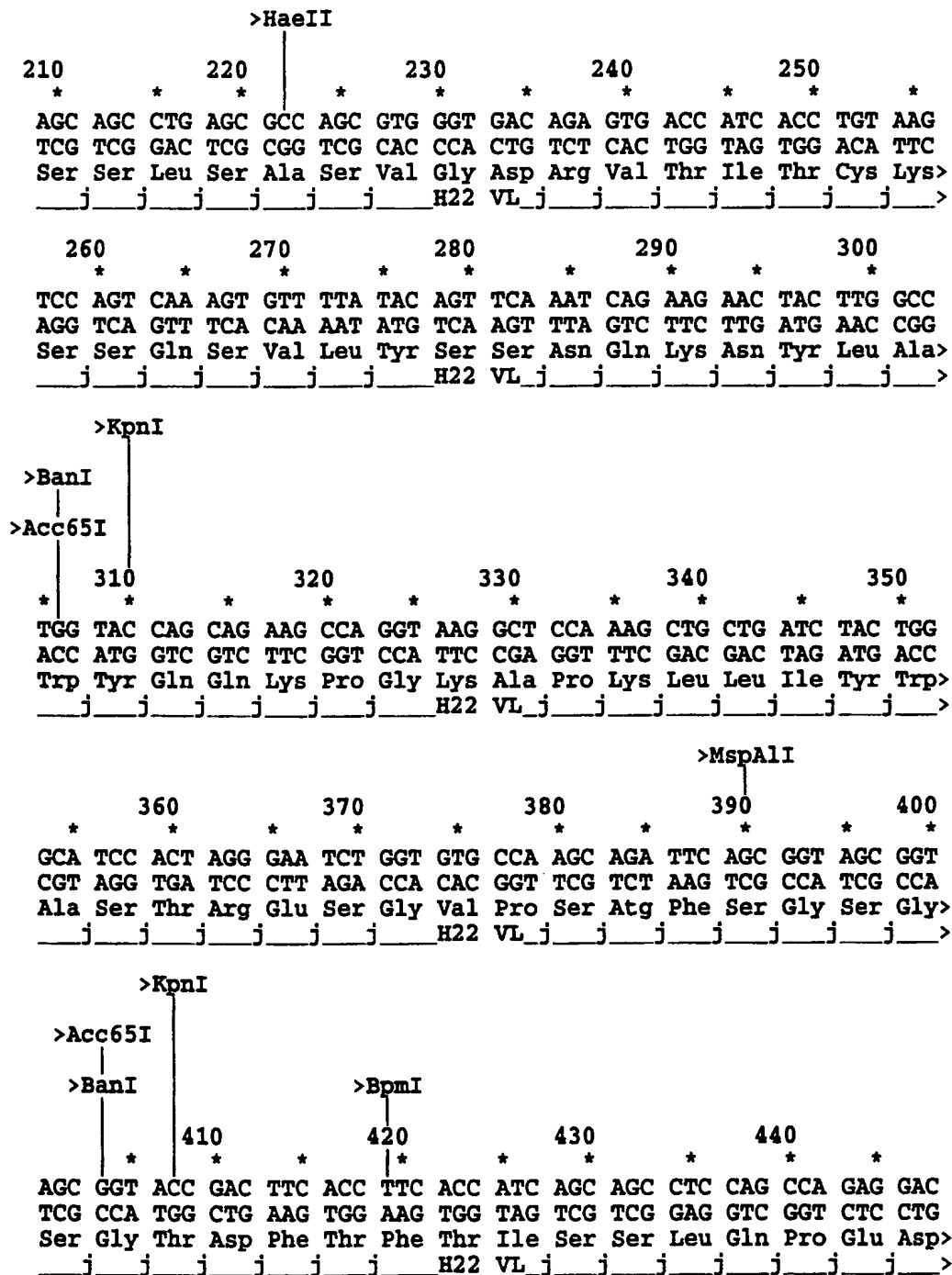


Fig. 4B

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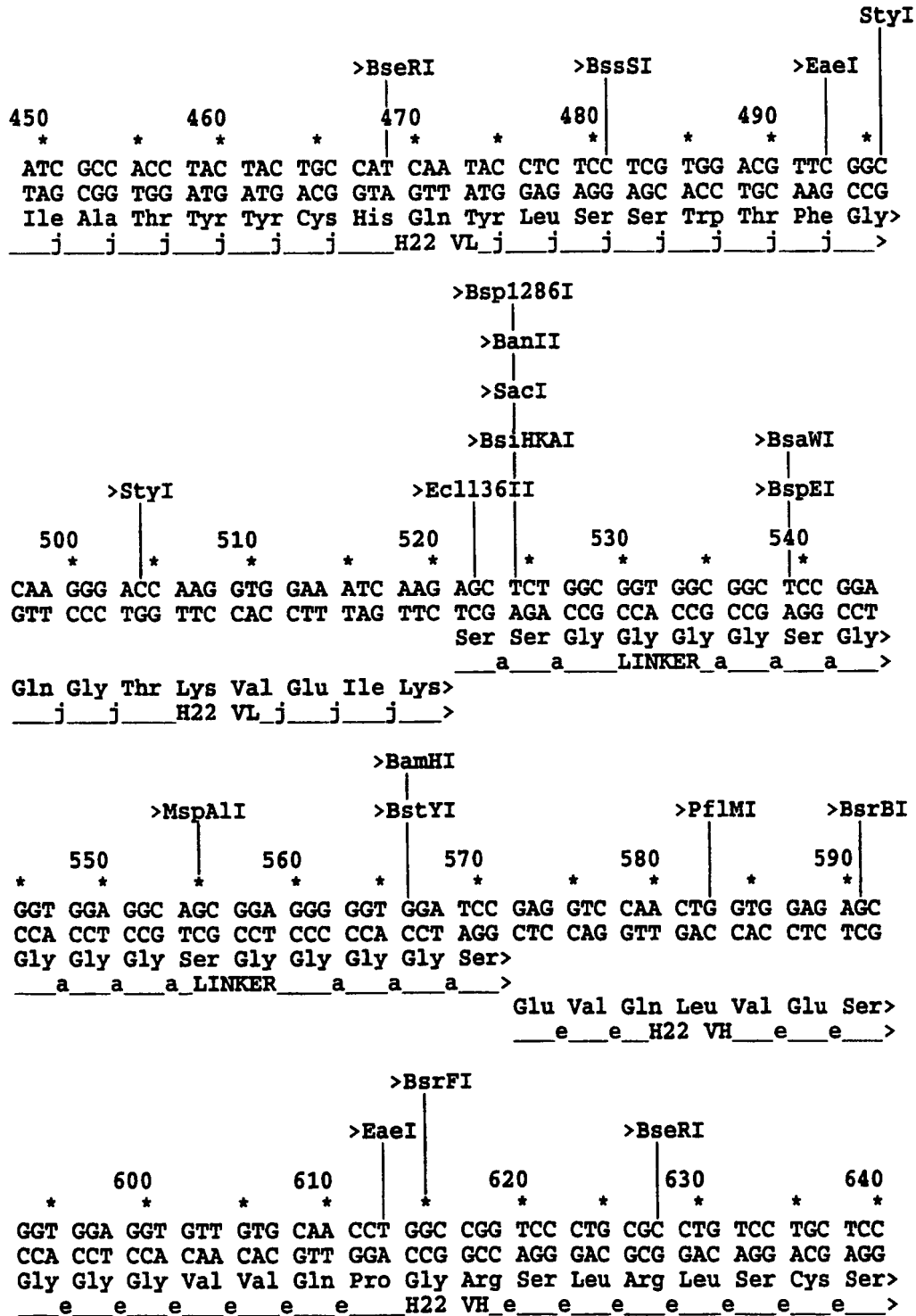


Fig. 4C

										>AflIII																				>BamI																			
650										660										670										680																			
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TCG	TCT	GGC	TTC	ATT	TTC	AGT	GAC	AAT	TAC	ATG	TAT	TGG	GTG	AGA	CAG	AGC	AGA	CCG	AAG	TAA	AAG	TCA	CTG	TTA	ATG	TAC	ATA	ACC	CAC																				
Ser	Ser	Gly	Phe	Ile	Phe	Ser	Asp	Asn	Tyr	Met	Tyr	Trp	Val	Arg	Gln																																		
_e_e_e_e_e_e_e_e_e_e										H22										vh_e_e_e_e_e_e_e_e_e_e																													
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690										700										710										720										730									
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*																				
GCA	CCT	GGA	AAA	GGT	CTT	GAG	TGG	GTT	GCA	ACC	ATT	AGT	GAT	GGT	GGT	CGT	GGA	CCT	TTT	CCA	GAA	CTC	ACC	CAA	CGT	TGG	TAA	TCA	CTA																				
Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	ala	Thr	Ile	Ser	Asp	Gly	Gly																																		
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_e_e_e_e_e_e_e_e_e_e										E										H22										VH_e_e_e_e_e_e_e_e_e_e																			
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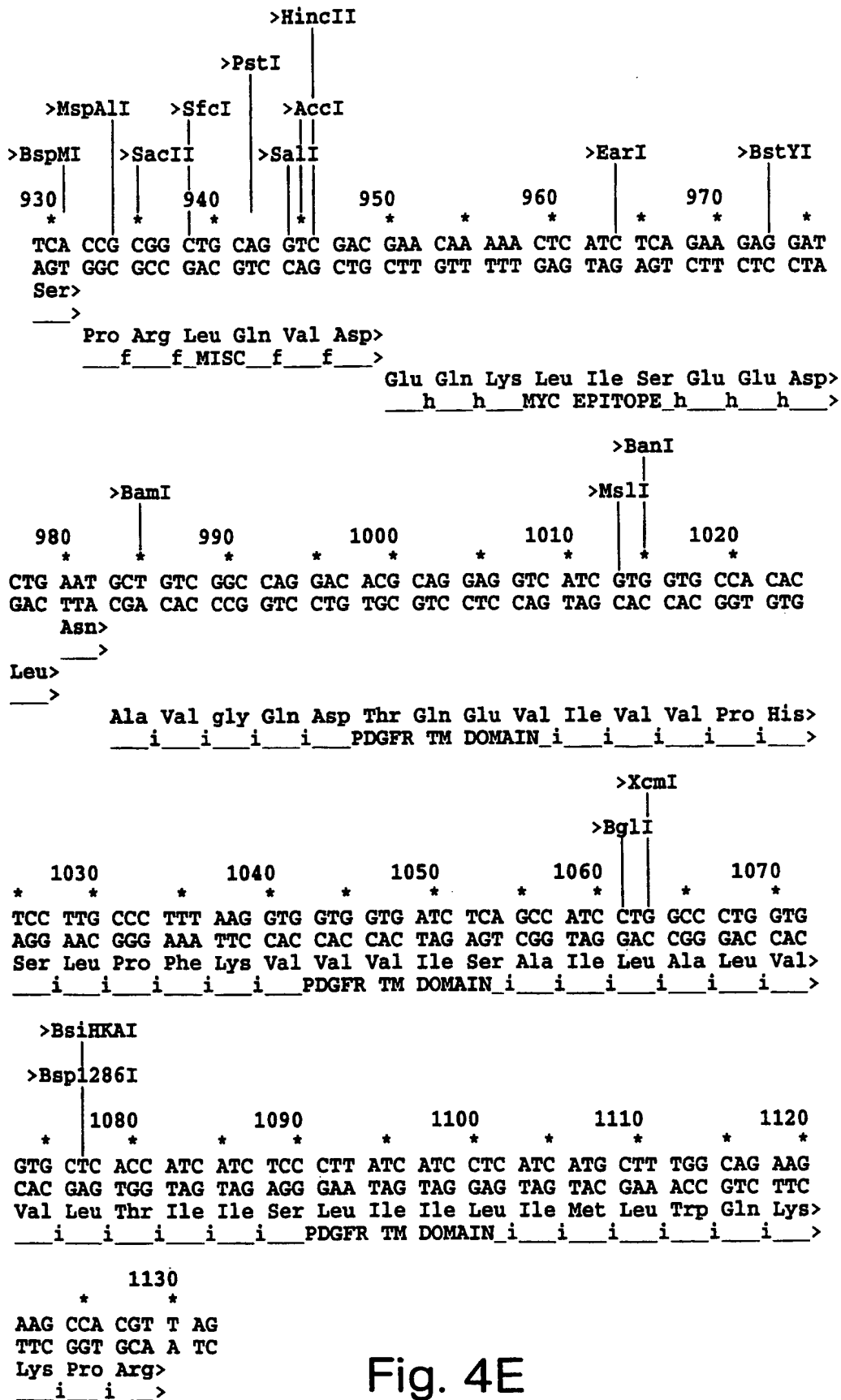


Fig. 4E

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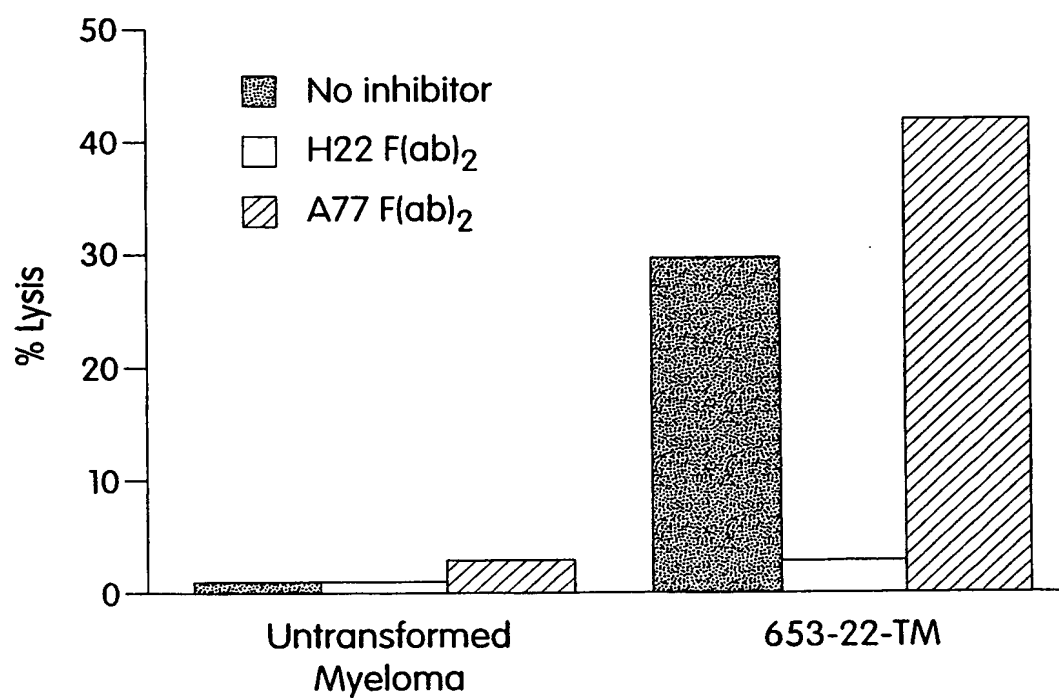


Fig. 5

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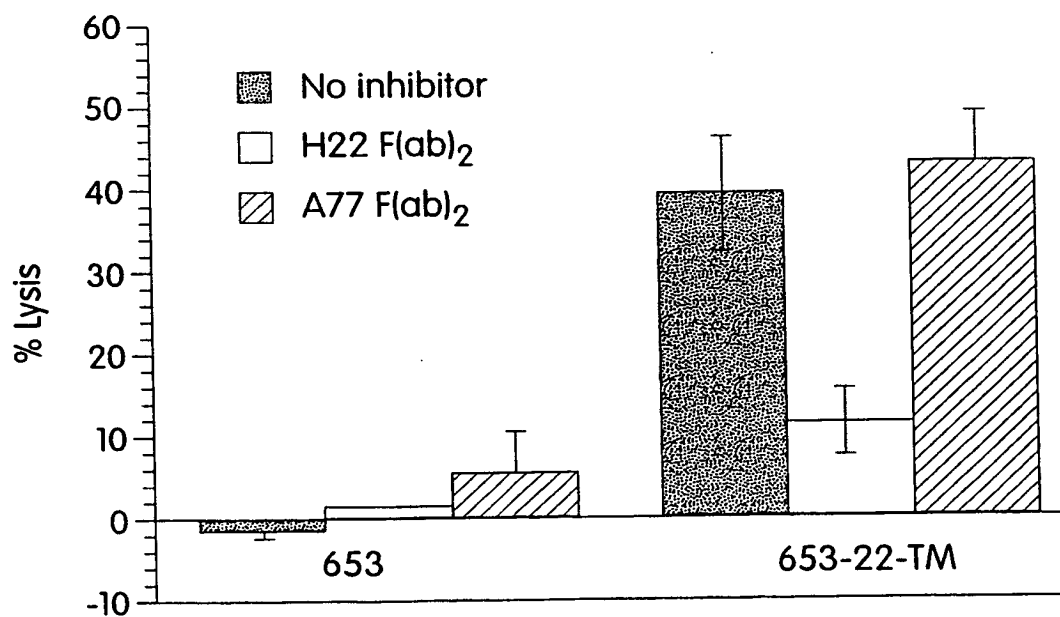


Fig. 6

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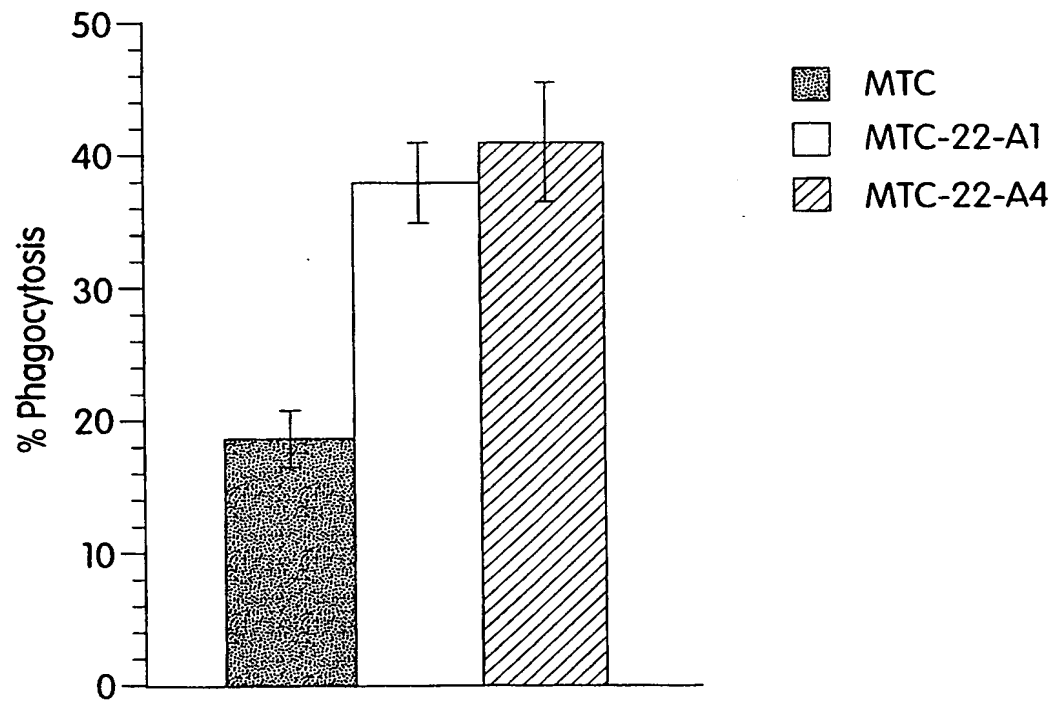


Fig. 7

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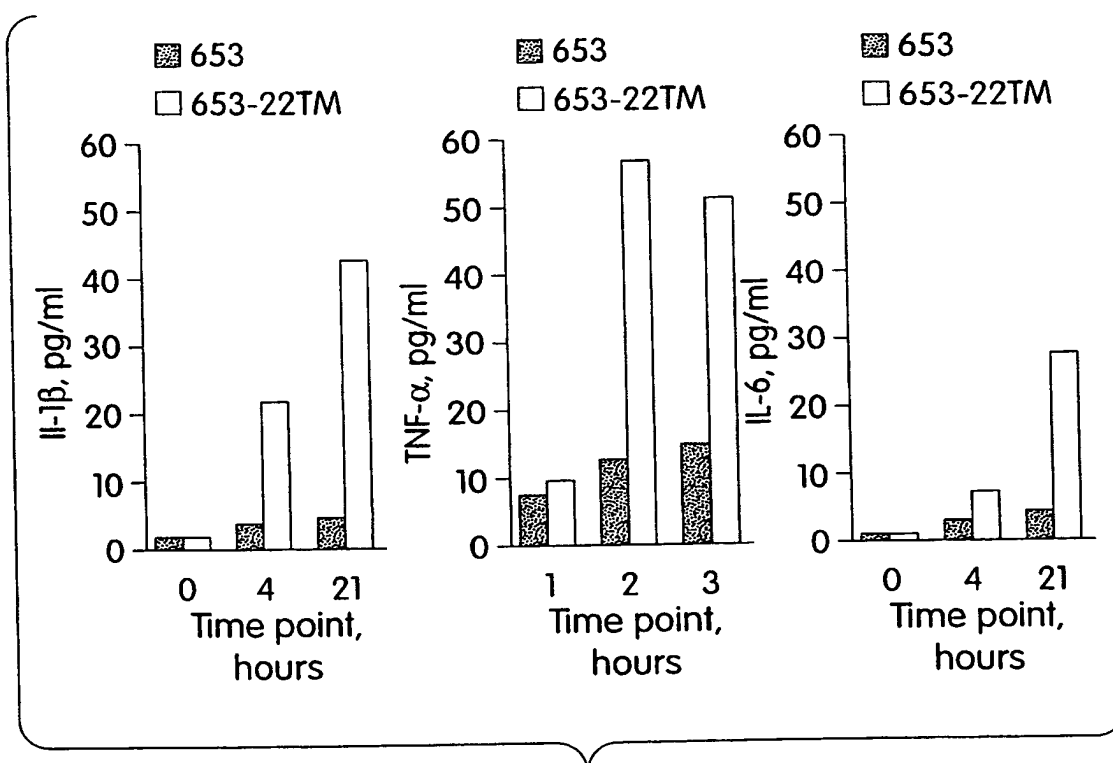


Fig. 8

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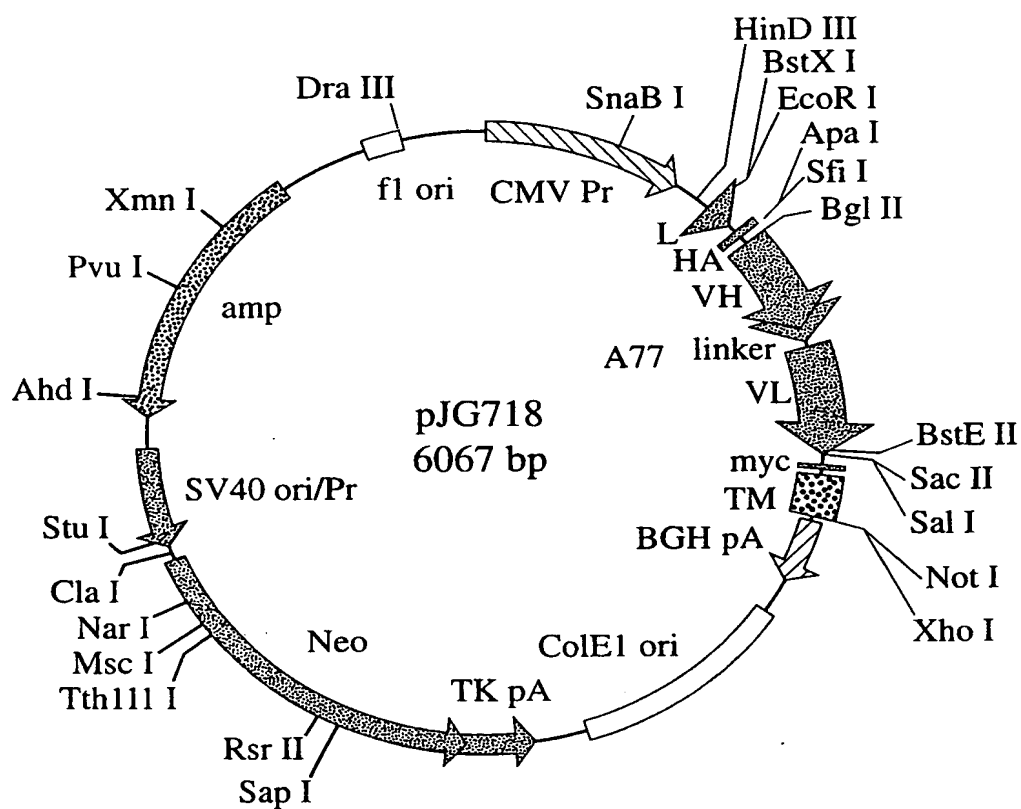


Fig. 9

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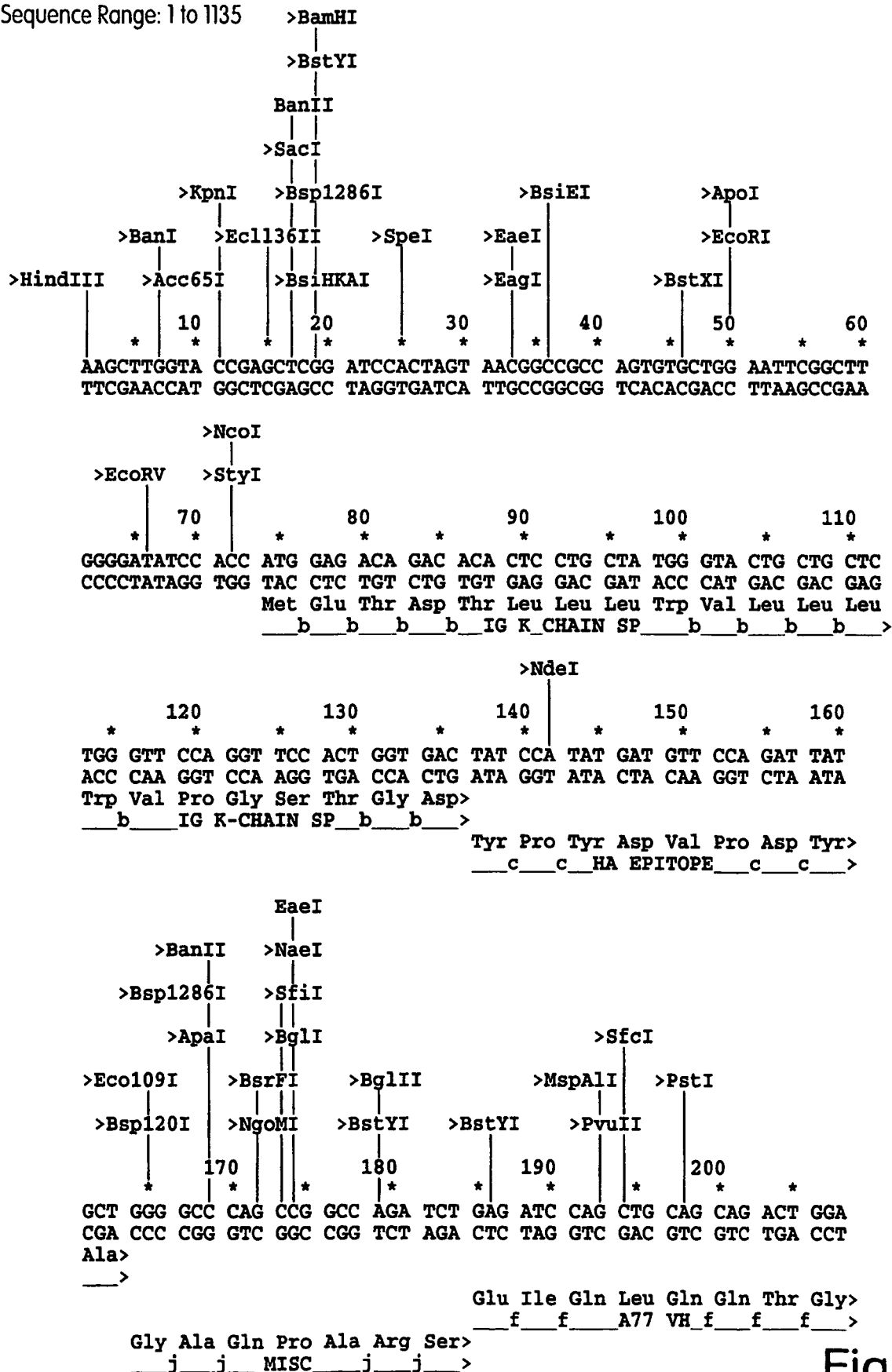


Fig. 10A

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Pro Glu Leu Val Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala>
_f _f _f _f _f _f _f _f A77 VH _f _f _f _f _f _f _f _f >

                                                    >NcoI
                                                    >StyI

260      *      *      270      *      280      *      290      *      300      *
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_f _f _f _f _f _f _f _f A77 VH _f _f _f _f _f _f _f _f >

                        >AseI
                        >SspI

310      *      *      320      *      330      *      340      *      350      *
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_f _f _f _f _f _f _f _f A77 VH _f _f _f _f _f _f _f _f >

                                                    >AccI
>ScaI
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  >DrdI
  >SfcI
450      *      *      460      *      470      *      480      *      490      *
GAG GAC TCT GCA GTC TAT TAC TGT GTA AGA GGA GTT TAT TAC TAC GGT
CTC CTG AGA CGT CAG ATA ATG ACA CAT TCT CCT CAA ATA ATG ATG CCA
Glu Asp Ser Ala Val Tyr Tyr Cys Val Arg Gly Val Tyr Tyr Tyr Gly>
_f _f _f _f _f _f _f _f A77 VH _f _f _f _f _f _f _f _f >

```

Fig. 10B

>StyI

>BpmI

> ApoI

>Bsp1286I

>BS1HKAI

>ApaLI

>BanI

>HincII

>PstI

>MspA1I

>Sfci

>ACCI

>BspMI

>SacII

>Sali

>EarI

>BstYI

Ile Lys>

g >

Pro Arg Leu Gln Val Asp>

h h MISC h h >

>BanI

>Ms1i

Ala Val Gly Gln Asp Thr Gln Glu Val Ile Val Val Pro>

e e e e PDGFR TM DOMAIN e e e e >

Asn>

Fig. 10D

SUBSTITUTE SHEET (RULE 26)

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```

                                >XcmI
                                |
                                >Bg1I
                                |
      1030      1040      1050      1060      1070
      *        *        *        *        *
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GTG AGG AAC GGG AAA TTC CAC CAC CAC TAG AGT CGG TAG GAC CGG GAC
His Ser Leu Pro Phe lys Val Val Val Ile Ser Ala Ile Leu Ala Leu>
__e__e__e__e__e__e__PDGFR TM DOMAIN__e__e__e__e__e__e__>

      >BsiHKAI
      |
      >Bsp1286I
      |
      1080      1090      1100      1110      1120
      *        *        *        *        *
GTG GTG CTC ACC ATC ATC TCC CTT ATC ATC CTC ATC ATG CTT TGG CAG
CAC CAC GAG TGG TAG TAG AGG GAA TAG TAG GAG TAG TAC GAA ACC GTC
Val Val Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln>
__e__e__e__e__e__e__PDGFR TM DOMAIN__e__e__e__e__e__e__>

      1130
      *        *        *
AAG AAG CCA CGT TAG
TTC TTC GGT GCA ATC
Lys Lys Pro Arg>
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Fig. 10E

19/19

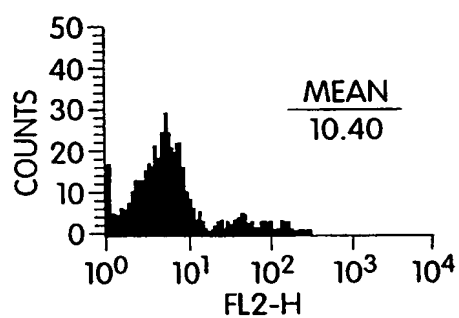


Fig. 11A

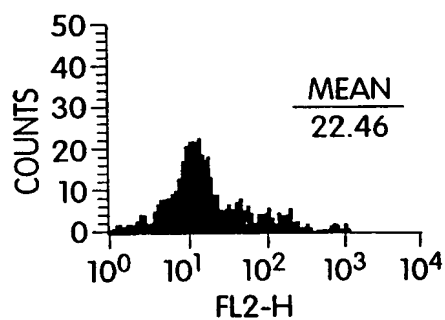


Fig. 11B

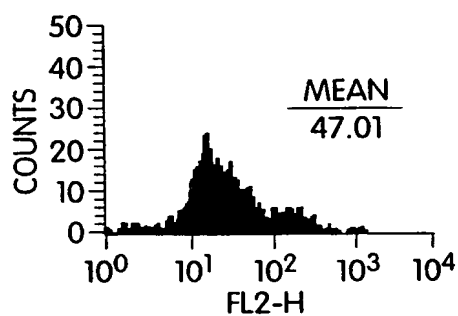


Fig. 11C

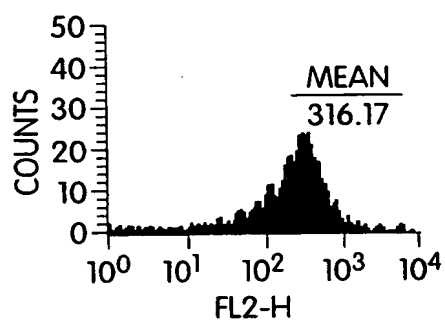


Fig. 11D

- 1 -

SEQUENCE LISTING

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```

```

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      80              85              90

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- 2 -

Trp	Ala	Ser	Thr	Arg	Glu	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser		
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ggt	agc	ggt	acc	gac	ttc	acc	ttc	acc	atc	agc	agc	ctc	cag	cca	gag	445	
Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	Thr	Ile	Ser	Ser	Leu	Gln	Pro	Glu		
	110					115				120							
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Asp	Ile	Ala	Thr	Tyr	Tyr	Cys	His	Gln	Tyr	Leu	Ser	Ser	Trp	Thr	Phe		
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Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	Ser	Leu	Arg	Leu	Ser	Cys		
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Tyr	Glu	Gly	Ala	Met	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	Pro	Val	Thr	Val		
	270					275					280						
tcc	tca	ccg	cgg	ctg	cag	gtc	gac	gaa	caa	aaa	ctc	atc	tca	gaa	gag	973	
Ser	Ser	Pro	Arg	Leu	Gln	Val	Asp	Glu	Gln	Lys	Leu	Ile	Ser	Glu	Glu		
285					290					295					300		
gat	ctg	aat	gct	gtg	ggc	cag	gac	acg	cag	gag	gtc	atc	gtg	gtg	cca	1021	
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				305					310					315			

- 3 -

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gtg gtg ctc acc atc atc tcc ctt atc atc ctc atc atg ctt tgg cag 1117
Val Val Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp Gln
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Tyr Ala Gly Ala Gln Pro Ala Arg Ser Glu Ile Gln Leu Gln Gln Thr
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- 4 -

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Gly Ser Ser Tyr Glu Ala Phe Pro Tyr Trp Gly Gln Gly Thr Leu Val			
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Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly			
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Pro His Ser Leu Pro Phe Lys Val Val Val Ile Ser Ala Ile Leu Ala			
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- 5 -

Leu Val Val Leu Thr Ile Ile Ser Leu Ile Ile Leu Ile Met Leu Trp
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cag aag aag cca cgt tag 1135
Gln Lys Lys Pro Arg
350



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 5/22, C07K 16/28, A61K 39/00 // C12N 15/62, C07K 19/00	A3	(11) International Publication Number: WO 99/28349 (43) International Publication Date: 10 June 1999 (10.06.99)
(21) International Application Number: PCT/US98/25556 (22) International Filing Date: 2 December 1998 (02.12.98) (30) Priority Data: 60/067,232 2 December 1997 (02.12.97) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/067,232 (CIP) Filed on 2 December 1997 (02.12.97) (71) Applicant (for all designated States except US): MEDAREX, INC. [US/US]; 1545 Route 22 East, P.O. Box 953, Annandale, NJ 08801-0953 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): KELER, Tibor [US/US]; 30 Park Road, Ottsville, PA 18942 (US). GOLDSTEIN, Joel [US/US]; 3804 Hana Road, Edison, NJ 08817 (US). GRAZIANO, Robert [US/US]; 1046 County Road, Route 519, Frenchtown, NJ 08825 (US). DEO, Yashwant, M. [US/US]; 3009 Peacock Drive, Audubon, PA 19403 (US).	(74) Agents: REMILLARD, Jane, E. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 18 November 1999 (18.11.99)	
(54) Title: CELLS EXPRESSING ANTI-Fc RECEPTOR BINDING COMPONENTS (57) Abstract <p>Cells transformed to express on their surface a component which binds to an Fc receptor of an effector cell are disclosed. Also disclosed are expression vectors used to transform the cells. Once transformed, the cells bind to effector cells via the Fc receptor of the effector cell to stimulate an effector cell mediated immune response.</p>		

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DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/25556

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 5/22, C07K 16/28, A61K 39/00 // C12N 15/62, C07K 19/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, MEDLINE, SCISEARCH, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9720048 A2 (BRISTOL-MYERS SQUIBB COMPANY), 5 June 1997 (05.06.97), page 6, line 9 - page 13, line 30 --	1-10,14-17, 22,25,27, 31-35,38-40, 42,44,45
X	DE 19634159 C1 (GSF - FORSCHUNGSZENTRUM FÜR UMWELT UND GESUNDHEIT GMBH), 25 Sept 1997 (25.09.97), See esp. column 1, lines 43-47, 57-64; column 3, lines 54-56; claims --	1-11,14-16, 27-30
X	WO 9735004 A1 (MEDICAL RESEARCH COUNCIL), 25 Sept 1997 (25.09.97), page 3, line 17 - page 7, line 16 --	1-8,14-17, 22,27,40,42, 44,45



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 July 1999

Date of mailing of the international search report

13.09.99

Name and mailing address of the International Searching Authority
European Patent Office P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel(+31-70)340-2040, Tx 31 651 epo nl,
Fax(+31-70)340-3016

Authorized officer

HAMPUS RYSTEDT/E1s

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/25556

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9215322 A1 (THE GENERAL HOSPITAL CORPORATION), 17 Sept 1992 (17.09.92), page 9, line 22 - page 14, line 21, claims 11,18-20 --	1-8,14-17, 22,27,40,42, 44,45
X	WO 9210591 A1 (CELL GENESYS, INC.), 25 June 1992 (25.06.92), page 4, line 26 - page 9, line 6; page 14, line 16 - page 17, line 2 --	1-8,14-17, 22,27,40,42, 44,45
A	US 5635600 A (MICHAEL W. FANGERET AL), 3 June 1997 (03.06.97), See esp. column 2, line 32- line 33 --	1-16,19,20, 28-39
A	WO 9205793 A1 (MEDAREX, INC.), 16 April 1992 (16.04.92), See esp. page 3, line 1 - line 5 -- -----	9,12,13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 25556

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

The application relates to five inventions:

1. A cell expressing an Fc-receptor binding component on its surface and use thereof, according to claims 17-18, 21, 28-30 (all partially), 1-8, 14-16, 19-20, 27, 31-32 (all completely).
2. A cell expressing an Fc-receptor binding component and a tumour antigen on its surface, according to claims 31, 33-39 (all partially), 9-11 (all completely).
3. A cell expressing an Fc-receptor binding component and a pathogen antigen on its surface, according to claims 9, 33-39 (all partially), 12-13 (all completely).
4. A method of increasing an immune response comprising administration of a cell according to invention 1 and an agent increasing Fc-receptor expression, according to claims 28-30.
5. A fusion protein comprising a transmembrane protein and an Fc-receptor binding protein, an expression vector encoding the fusion protein and a cell expressing it, according to claims 17-18, 21, 38 (partially), 22-26, 40-45 (all completely).

The technical feature characterising invention 1 is the exposure of a Fc-receptor binding component on the surface of a cell. This feature is known in the prior art, see e.g. DE, 19634159, C1. Inventions 2 and 3 relate to a cell according to invention 1, further comprising antigens exposed on the cell surface. The technical feature characterising invention 2 is thus a tumour antigen exposed on the cell surface and the technical feature characterising invention 3 is a cell with a pathogen antigen exposed on the cell's surface. Invention 4 relates to a method using the cell of invention 1. The technical feature characterising invention 4 is the administration of a cell of invention 1 together with an agent increasing Fc-receptor expression. Since the technical feature shared by inventions 2, 3 and 4, i.e. the technical feature of invention 1, is previously known, inventions 2, 3 and 4 do not form a single general inventive concept over the prior art as required by PCT Rule 13.1.

Invention 5 relates to a fusion protein, characterised by the technical feature that it comprises an Fc-receptor binding domain and a transmembrane domain. The protein of invention 5 can be used within the scope of invention 1, but its use is not restricted to invention 1. It is therefore not considered to be a part of the general concept defined by invention 1 and consequently does not fulfil the requirements of PCT Rule 13.1.

Invention 1 has been searched. It was considered that the additional search of inventions 2, 3 and 4 did not justify an additional fee, and they have been incorporated in the partial search. However, a search of invention 5 requires one additional fee.

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